MICROCOCCI IN FERMENTED MEAT PRODUCTS

CLASSIFICATION AND DESCRIPTION OF 171 DIFFERENT STRAINS

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CLASSIFICATION AND DESCRIPTION OF 171 DIFFERENT STRAINS

BY
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TO BE PRESENTED, WITH THE PERMISSION OF THE FACULTY OF AGRICULTURE AND FORESTY OF THE UNIVERSITY OF HELSINKI, FOR PUBLIC CRITICISM IN AUDITORIUM III (PORTHANIA), ON DECEMBER 17TH, 1960, AT 12 O'CLOCK NOON.

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Preface

The present investigation was carried out in the years 1958—1960 in the Research Laboratory of the Farmers' Cooperative Packinghouses, Hämeenlinna. I wish to express my deep gratitude towards the Directors of the Research Laboratory for the opportunity and facilities to accomplish this work, and to Docent FRITZ P. NIINIVAARA, *D. Sc., Head of the Research Laboratory, for the great interest he has shown in my work and for all his help.*

It is with pleasure that I give respectful expression to my obligation towards Professor UNTO VARTIOVAARA, *D. Sc., Head of the Department of Microbiology of the University of Helsinki, for the unfailing interest displayed by him and for valuable remarks concerning my work.*

My sincerest thanks are due to Docent HELGE GYLLENBERG, *D. Sc., to whom I am indebted for criticism and encouragement throughout the course of this investigation.*

All my colleagues in the Research Laboratory are also deserved of my warmest thanks, in particular Mrs. MARJATTA KREUZER, *M. Sc., and Mrs.* KERTTU TURIAINEN, *who have assisted me in the laboratory work.*

Mr. ULJAS ATTILA, *M. Sc., has translated the manuscript into English, for which I wish to thank him.*

Finally I wish to mention in grateful acknowledgement the courtesy shown me by the Scientific Agricultural Society of Finland by inclusion of this study in its series of publications, and the financial help furnished by a grant received from the U.S. Department of Agriculture, Agricultural Research Service, Washington, U.S.A.

Hämeenlinna, November 20th, 1960.

Matti S. Pohja

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I. Introduction

Previous bacteriological investigation of meat products has been centered mainly on the organisms responsible for diseases of domestic animals, deterioration phenomena, and food poisoning. This is thought to be due to the fact that the clear manifestation of such phenomena has rather forcefully allured the investigators to clarify their causes. It can be said, indeed, that the bacteriological investigations of the meat branch have born the hall-mark of veterinary hygienics; not until quite lately has attention been paid in several countries to the bacteriology of meat products from the technological point of view. These recent studies have also placed emphasis on the bacteriological background of the phenomena resulting in the production of a number of desirable characteristics in the ripening process of certain meat products. First among these so-called useful bacteria to receive attention with respect to their action and to their favourable effect on the formation of flavour in certain meat products were the lactobacilli (JENSEN and PADDOCK 1940). Superior to the lactobacilli in their versatility as producers of desirable characteristics are the micrococci; their contribution to the improvement of colour, āroma, flavour and keeping quality of so-called dry sausages (Salami-type sausages) was first investigated by NIINIVAARA (1955); later by NIINIVAARA and POHJA (1957c) and by POHJA (1960). The results of these investigations warrant the opinion that the micrococci constitute in fact the most important group of bacteria in regard to their activity in the processing of dried meat products. This conclusion has induced the author to subject this particular group of bacteria to closer investigation and characterization.

In the author's previous investigations (POHJA 1960), a selection procedure for micrococci has been developed, by which the strains suitable for use in the processing of dry sausages can be selected from a great multitude of micrococci by means of a few simple tests only. The experimental material consisted of selected micrococci isolated from various kinds of dry sausages and from ham-curing brines,

Among 702 isolated micrococcus strains, only three strains were marked out by the selection procedure as strains that could be considered useful in the manufacturing of dry sausages. At an attempt to identify these »meat micrococci» with the species described in the conventional taxonomic reference books (e.g. Bergey's Manuals 1948, 1957; KRASILJNIKOV 1949; WILSON and MILES 1955) considerable overlapping of characteristics was noted. It appears obvious, therefore, that the micrococci, and particularly the micrococci isolated from meat products, cannot with a satisfactory accuracy for technological purposes be identified by means of the descriptions of species and groups presented in the manuals.

Recent studies concerning the classification of micrococci have shown that their subdivision into groups should be done on an entirely different basis from that presented, e.g., in Bergey's Manuals. The investigations of ABD-EL-MALEK and GIBSON (1948), SHAW et al. (1951) and HILL (1959) in particular have been instrumental in creating a basis for our new concepts concerning the classification of micrococci. ABD-EL-MALEK and GIBSON have mainly studied cocci derived from dairy products, while cocci from various sources made up the materials investigated by SHAW et al., and by HILL. It seems that these collections did not include any actual meat micrococci and it is therefore rather to be expected that the types described by the said authors will not suffice as such for the classification of the meat micrococci.

The principal object with the investigations carried out by the present author was to establish a basis for the grouping of the micrococci encountered in meat products and to elucidate the following questions:

(a) Are there such types among the micrococci derived from meat products which have not been described before?

(b) If the meat micrococci can be grouped in an expedient manner, then how will this classification be consistent with the opinions previously presented concerning the grouping principles of the micrococci?

The experimental material of the present work consists of 171 strains chosen among the micrococci isolated in the author's previous investigations (POHJA 1960), all of which were mutually different with respect to one or another characteristic. An attempt was made to group these strains, at first, according to the system presented in Bergey's Manuals (1948, 1957), then according to those of SHAW et al. (1951) and of HILL (1959); and finally the so-called Adansonian

method of classification elaborated by SNEATH (1957a, b) was applied to the present material. The results obtained by this latter procedure were applied in an attempt to complement the grouping system of micrococci given by SHAW et al., and by HILL, so that they might also encompass the meat micrococci.

Finally, the three micrococcus strains selected by means of the selection procedure outlined in the foregoing (POHJA 1960), which could be considered suitable for use in the manufacturing of dry sausage, were placed in the grouping system obtained in this manner. This was done in order to ascertain whether these micrococci, regarded as the most useful from the standpoint of meat technology, constitute a separate subgroup in the system developed in this work.

II. Review of the taxonomy and grouping of micrococci

1. Definition of genus *Micrococcus*

The arrangement of the cells is considered a taxonomically significant characteristic of spherical bacteria. For this reason within the family *Micrococcaceae*, the diplococci, tetracocci and sarcinae have usually been distinguished as separate from micrococci on account of the fact that the cells of the first-mentioned remain together in pairs, tetrads or packets after division, whereas micrococci are segregated into separate cells or accumulate in irregular clusters. However, it is difficult in some instances to distinguish among the Gram-positive, catalase-positive cocci between micrococci, diplococci, tetracocci and sarcinae.

In Bergey's Manuals (1948, 1957), the aerobic and facultatively anaerobic representatives of the *Micrococcaceae* family have been divided on the basis of their characteristic arrangement of the cells into the genera *Micrococcus* (*Staphylococcus*), *Gaffkya* and *Sarcina*. SHAW et al. (1951) have placed the aerobic and facultatively anaerobic Gram-positive, catalase-positive cocci of the said genera in one and the same genus, *Staphylococcus*. HILL (1959) has suggested that, among the cocci described by SHAW et al., the coagulase-positive, acetoin-positive and fermentative cocci (the species *Staphylococcus aureus*, *S. saprophyticus* and *S. lactis* according to SHAW et al.) should be referred to the genus *Staphylococcus* and those forming red pigment, as well as the non-fermentative cocci (species *S. roseus* and *S. afermentans* of SHAW et al.) to the genus *Micrococcus*.

In the isolation of micrococci done in the present work, the aim has been to obtain an ultimate experimental material comprising exclusively catalase-positive, Gram-positive cocci, the cells of which occur separately or in irregular clusters. This implies that such strains were eliminated which were manifestly observed to be diplococci,

tetracocci or sarcinae. It is still possible, however, that some strains of the diplococcus or tetracoccus type may have remained among those selected to constitute the present experimental material. Of the concepts suggested by HILL (1959), only the coagulase-positive and the acetoin-producing cocci have been accepted as belonging to the genus *Staphylococcus* (that is, the species *S. aureus* and *S. saprophyticus* of SHAW et al.), and both the fermentative and non-fermentative and the pink pigment-forming cocci mentioned by HILL have been referred to the genus *Micrococcus*.

2. Differentiation between genera *Micrococcus* and *Staphylococcus*

One of the central controversies in the taxonomy of micrococci has been that whether the genus should be called *Micrococcus* or *Staphylococcus*. Numerous investigators have assumed that *Micrococcus* COHN 1872 and *Staphylococcus* ROSENBACH 1884 constitute two mutually differentiable genera, but evidence in support of this contention seems to be lacking (Editorial Board of Int. Bull. Bact. Nom. and Tax. 1954).

WINSLOW and WINSLOW (1908) have tried to distinguish the more parasitic, orange-coloured and white staphylococci from the more saprophytic, yellow and red micrococci. HUCKER (1924a) and RAHN (1929) have not recognized this opinion. HUCKER (1924a), having studied a collection of 316 strains, states that there is no correlation of characteristics by which an unaffected subgrouping of this group could be achieved. However, HUCKER (1928) has divided the cocci agglomerating in clusters into 18 species. His differentiation was based on formation of pigment, liquefaction of gelatine, nitrate reduction and utilization of ammonium phosphate as sole source of nitrogen. This has been adopted, with only minor alterations, in the sixth edition of Bergey's Manual (1948), where all aerobic and anaerobic micrococci have been referred to the genus *Micrococcus* and the name *Staphylococcus* has been entirely discarded.

VAN ESELTINE (1955) mentions that »the micrococci cannot be considered as constituting more than one genus. Whether this genus should be called *Micrococcus* or *Staphylococcus* is debatable, although *Micrococcus* COHN 1872 clearly has priority and would appear to be the name of choice provided only that a suitable culture can be found to conform to the description of the type species, *Micrococcus luteus*».

ABD-EL-MALEK and GIBSON (1948), in their investigation of micrococci isolated mainly from milk, have divided them into three groups. Their Group I comprises sugar-fermenting parasites, which are comparatively sensitive to heat. These cocci have been referred to staphylococci. The group can further be subdivided into four subgroups, in two of which acetoin is produced from glucose. These cocci are fermenters of mannitol, but they are coagulase-negative. Group III contains saprophytic thermoduric sugar-fermenters, which are typical »dairy micrococci». The cocci in Group II are obligatorily aerobic cocci, which do not produce acid from glucose. They represent an intermediate form between Groups I and III. ABD-EL-MALEK and GIBSON consider the distinction between the genera *Micrococcus* and *Staphylococcus* indefinite.

According to EVANS et al. (1955), separation of the genera *Micrococcus* and *Staphylococcus* would be desirable as well as feasible. It should be based on the behaviour of the organisms with respect to oxygen when they are growing on a standard substrate containing glucose. The species which produce acid from glucose also under anaerobic conditions should be referred to genus *Staphylococcus* and the obligatorily aerobic species to genus *Micrococcus*. This is also the principle underlying the distinction between *Micrococcus* and *Staphylococcus* in the seventh edition of Bergey's Manual (1957). However, no evidence in support of this differentiation has been presented in the Manual (cf. also SKERMAN 1959). The species producing acid under aerobic conditions would thus belong to genus *Micrococcus*, and those showing this behaviour also in anaerobic conditions, to genus *Staphylococcus*. In the said Manual, the obligatorily anaerobic cocci have been transferred to a genus of their own, *Peptococcus*.

In the opinion of SHAW et al. (1951), there is no motivation for continued grouping of these cocci as the genera *Staphylococcus*, *Micrococcus*, *Gaffkya* and *Sarcina*, and they have accordingly placed all aerobic, Gram-positive, catalase-positive cocci in the genus *Staphylococcus*. They restrict the generic name *Sarcina* to obligatorily anaerobic cocci alone. Adoption of the name *Staphylococcus* is advocated by them on the grounds that the first definition of the genus *Micrococcus* COHN 1872 did not sufficiently circumscribe it, leaving the possibility of referring to this genus also rod-shaped bacteria. By the rules of taxonomic practice (e.g. BUCHANAN et al. 1948), the generic name *Staphylococcus* would therefore have priority over *Micrococcus*.

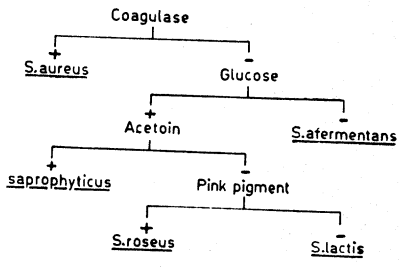
SHAW et al. (1951) refer to genus *Staphylococcus* the five species *S. aureus*, *S. saprophyticus*, *S. lactis*, *S. roseus* and *S. afermentans*, differentiating the species by means of four simple tests, namely, the coagulase test, the production of acetoin from glucose, the formation of pink pigment and the production of acid from glucose. The coagulase-positive cocci belong to species *S. aureus*, those producing acetoin to *S. saprophyticus* and the pink pigment-forming cocci to species *S. roseus*. The cocci forming neither acetoin nor red pigment belong to species *S. lactis* and those producing no acid from glucose, to *S. afermentans*. It is likely that the acetoin-producing strains in Group I presented by ABD-EL-MALEK (1948) belong to the species *S. saprophyticus* of SHAW et al. and his Group II belongs to *S. afermentans*.

HILL (1959) has mostly employed in his investigations the same strains which were studied by SHAW et al.; he divided them into groups by the Adansonian classification system elaborated by SNEATH (1957a, b). HILL interpreted his results to the effect that among the five species named by SHAW et al., *S. aureus*, *S. saprophyticus* and *S. roseus* are natural groups, whereas he denies this for *S. lactis* and *S. afermentans*. This contention is based on the fact that the individual strains referred to the two last-mentioned species are frequently mutually as much different as they are from strains belonging to other species. According to HILL, the group of the micrococci could be divided into two main parts, which might be considered as separate genera. He suggests, indeed, that the generic name of *S. roseus* and of the cocci which do not produce acetoin nor acid from glucose should be *Micrococcus*. He refers to the *S. lactis* group of SHAW et al. as »fermentative miscellaneous staphylococci» and to the *S. afermentans* group as »non-fermentative miscellaneous micrococci».

The grouping schemes of SHAW et al. and of HILL are as follows:

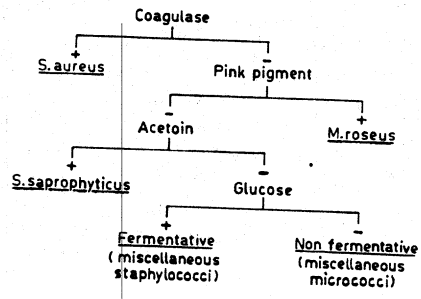
Grouping according to

SHAW et al. (1951)



Grouping according to

HILL (1959)



3. Characteristics previously employed as a basis for the groupin micrococci

One of the most centrally placed questions associated with t basis for the grouping of micrococci, and perhaps one of the m controversial, has been that of finding the properties by which furt grouping of the micrococci can be accomplished. A short review v be given here of the most important investigations that have b presented bearing on this matter.

Coagulase test. EVANS (1948), EVANS and NIVEN (1950) and EVA et al. (1955) state that »the coagulase-positive staphylococci repres one of the most homogeneous species of bacteria to be found in a genus». They have shown that a distinct correlation exists between t formation of coagulase and the mannitol-fermenting ability. Th has also been taken into consideration in the seventh edition Bergeys's Manual (1957) in the grouping of the species of gen *Staphylococcus* into two groups.

COWAN (1938) and SHAW et al. (1951) claim that the toxigeni pathogenic cocci can be distinguished from the non-pathogenic b means of the coagulase test. It should be noted, however, tha even though nearly all toxigenic cocci investigated so far hav proved to be coagulase-positive, all coagulase-positive strains do no form toxin (cf. GYLLENBERG 1958). THATCHER and SIMON (1956 have shown that also some coagulase-negative strains may b toxigenic. Coagulase-positivity can thus only be considered a »alarming» property, and it is not sufficient as ultimate evidence o toxigenicity (cf. GYLLENBERG 1958). It is therefore also obvious tha the coagulase test cannot be applied in differentiating between the genera *Staphylococcus* and *Micrococcus*, certainly not in the sense that the genus *Staphylococcus* would encompass all potentially patho genic coagulase-positive cocci, as then also the genus *Micrococcus* might contain pathogenic, but coagulase-negative, types.

Utilization of ammonium salts as sole source of nitrogen. HUCKER (1924a, b, 1928, 1948) mentions that the ability of micrococci to utilize ammonium salts would be a suitable basis for grouping. How ever, the opinion of VAN ESELTINE (1955) as well as EVANS et al. (1955) and SHAW et al. (1951) is that the ability of cocci to utilize ammonium nitrogen as their sole source of nitrogen is not a characteris tic of such constancy that it might be used in the subgrouping of these bacteria.

Fermentative reactions. CUMMINS and CUMMINS (1913) report that the fermentation tests were too unreliable and inconstant for use in the subgrouping of cocci. ANDREWES and GORDON (1907), DUDGEON (1908), JULIANELLE (1937), CHAPMAN et al. (1938) and others have employed the fermentation of mannitol as a basis for grouping. On the other hand, WINSLOW and WINSLOW (1908) and WINSLOW et al. (1920) dispute its significance. VAN ESELTINE (1955) claims that the production of acid from glucose, lactose and saccharose cannot be considered as a basis for grouping. BLAIR (1938) has found glucose fermentation to be one of the most constant fermentative reactions. ABD-EL-MALEK and GIBSON (1948) divided their strains into groups on the basis of their glucose fermentation. SHAW et al. (1951), too, accepted the glucose fermentation test and found that it provides a good starting point for subgrouping. On the other hand the mannitol-fermenting and lactose-fermenting ability of cocci is devoid of any noteworthy significance in their grouping.

Pigmentation. HUCKER (1924a, b, 1928) has considered the formation of pigment to be a species-differentiating property. However, ABD-EL-MALEK (1948) and VAN ESELTINE (1955) have observed that pigmentation is no constant characteristic. Among others, STEUER (1956) states that some compound in the substrate may have an inhibitory effect on the formation of pigment. For instance, he found that glycerol prevents the pigmentation of *Micrococcus pyogenes var. aureus*.

In the investigation of SHAW et al. (1951), pigmentation was found to be a variable occurrence in the *S. aureus* and *S. saprophyticus* groups, whereas it was fairly constant in the groups *S. lactis*, *S. roseus* and *S. afermentans*. Accordingly, SHAW et al. stated as their opinion that pigmentation may be used as a differentiating characteristic for the last-mentioned groups. The pink pigmentation of the *S. roseus* group has proved to be a comparatively constant characteristic, and no unpigmented variants have been observed in this group.

Other characteristics. HUCKER (1924 a, 1928) has also employed gelatine liquefaction and nitrate reduction in his grouping of the cocci. However, VAN ESELTINE (1955) has found that also hemolysis, gelatine liquefaction, ammonia production, nitrate reduction and the reaction in litmus milk cannot be used in the subgrouping of cocci. According to ABD-EL-MALEK and GIBSON (1948), acetoin production may be usable as a criterion particularly for coagulase-negative mannitol fermenters. They mention, moreover, that the production of ammonia from arginine, which was first introduced for the differen-

tiation of streptococci, promises to have applications among the micr cocci also. SHAW et al. (1951) consider also acetoin production property that might be utilized in the subgrouping of micrococci.

In the grouping of bacteria, usually some of their physiologic activities have been employed as a basis of grouping. A differe procedure was applied by CUMMINS and HARRIS (1955, 1956), wh studied the chemical composition of the cell wall, dividing the Gra positive, catalase-positive cocci into three groups on the basis their results, but this grouping is not consistent with any other syste that has been presented for these organisms. GREGORY and MABB (1957 a, b) have investigated the ninhydrin-positive compounds the unhydrolyzed extract from the cell walls; they, too, divided th cocci into three groups on the basis of their results, and their groupin is largely compatible with that presented by SHAW et al. (1951). appears in the light of these results that the grouping of bacter may also be based on the chemical composition of the cell, not on on the physiological capacities of the bacterium.

Summarizing the different foundations from which the grou ing of micrococci has been approached, the following can be sai The majority of the tests quoted in the manuals (e.g. Bergey's Manual 1948, 1957), by which a subgrouping of micrococci is effected, ar arbitrarily chosen. It appears quite likely in the light of presen knowledge that among the different physiological capacities of th cocci those specified by SHAW et al. (1951), namely, coagulase forma tion, pink pigmentation, acetoin and acid production from glucos are characteristics of fairly good constancy and can probably b employed in the subgrouping of cocci.

4. On the grouping of technologically usable meat micrococci

The contribution of bacteria in the processes resulting in formatio of taste, aroma and colour of the meat products has been recognized since the investigations of HOROWITZ-WLASSOWA (1931). However pure cultures of bacteria have not been used in the industrial manufacturing of meat products until after the investigation concerning dry sausages, carried out by NIINIVAARA (1955). His investigation presents the biochemical foundations of the use of such bacterial pure cultures in the processing of dry sausage and gives evidence of the practical results that can be achieved by this means. Subsequently several investigations of the same kind have been carried out also elsewhere, largely instigated by the positive results noted

in NIINIVAARA's work. Above all, the investigation of NIVEN (1955) have to be mentioned here. In their investigations, NIINIVAARA employed a certain *Micrococcus* strain, and NIVEN a *Pediococcus cerevisiae* strain. With the first-mentioned cultures the most notable achievements were more rapid formation of the desired colour, better taste and marked prevention of deterioration phenomena during the manufacturing process.

No other strains of bacteria except those just mentioned have been used in the industrial manufacturing of meat products so far. This can be attributed to the small amount of research that has been done in the entire field of meat technology and — more or less a corollary of this fact — the lack of a system for studying and testing bacteria that can be considered for such use.

All grouping systems having a bearing on micrococci which are encountered in the literature (e.g. Bergey's Manuals 1948, 1957; ABD-EL-MALEK and GIBSON 1948; KRASILJNIKOV 1949; SHAW et al. 1951; WILSON and MILES 1955; HILL 1959) were designed to provide a system by which the micrococci might be identified. None of these grouping systems in itself is suitable for the grouping of the particular micrococci which could be considered for use in dry sausage manufacturing. As a consequence, the selection of strains of bacteria with this purpose in mind has been more or less a matter of random trial and error and of good luck. There has been no exactly outlined system for assessing the suitability of various strains for use as agents in dry sausage manufacturing. In other words, further investigation bearing on the use of pure cultures of bacteria has lacked its necessary foundation as long as no consistent system for determining the properties of the strains had been developed for this purpose.

In his earlier investigations, the author has worked out a selection procedure (POHJA 1960) for the said micrococci, enabling its user to establish by means of a few tests whether some micrococcus strains are endowed with the characteristics desirable in a strain to be employed in the manufacturing of dry sausage. None of the micrococcus grouping systems presented up to date could be utilized in this work. It goes without saying that elaboration of the selection procedure had to be preceded by studies of the changes occurring in dry sausage during its ripening process and of the contribution of bacteria in bringing about the desired changes. The investigations of NIINIVAARA (1955) and of NIINIVAARA and POHJA (1956, 1957a, b, c) and numerous other investigations relating to the ripening of dry sausages (e.g. KELLER 1954; KELLER and MEYER 1954; NIVEN 1955; DEIBEL

1956; LERCHE 1956; CORETTI 1956 a, b; NIVEN et al. 1958; RAŠEI 1958) have been instrumental in elucidating the facts on which th said selection procedure is based.

It is obvious that the said selection procedure, intended to singl out strains suitable for use in the manufacturing of dry sausage cannot find use in the identification of micrococci. While the primar aim of the present work consists of developing a grouping for th micrococci occurring in meat and in meat products, by which th meat micrococci might be identified, it will still be interesting to se whether the particular micrococci found to be suitable for use in dr sausage manufacture belong to a given group in the grouping system A study to this effect will be presented subsequently in the Chapte »Discussion» (p. 68). In order to render these later consideration readily understandable, it is thought to be appropriate to give it this place a brief account of the micrococcus selection procedur developed by the author in his previous investigations (POHJA 1960)

5. The author's selection procedure for technologically usable mea micrococci

Altogether 702 micrococcus strains were isolated from dry sausage originating in eight different factories and from the brines used i curing dry sausages and hams. Among them, 380 strains were abl to grow on a substrate containing 5 % NaCl at pH 6.0 and at a cultivating temperature of 20°C. These conditions of salt concentration, pH and temperature were consistent with the conditions prevailing in dry sausage during its manufacturing process. The tests prescribed by the author's selection procedure were carried out with these 380 strains.

The first, most essential change in the ripening process of dry sausage is conversion of the grey colour of the sausage mix to red colour. As this reaction is dependent on reduction into nitrite of the nitrate added to the mix, a test revealing the nitrate reducing capacity of the micrococci has been chosen to constitute the first test in the series. 285 of the 380 strains were able to reduce nitrates to nitrites (Fig. 1).

Another factor influencing the formation of desired colour in dry sausages is the production of acid from carbohydrates. Further reduction of the nitrite produced from nitrate to nitrogen oxide occurs at increasing rate with lower pH in the sausage. The sausage mix should therefore contain bacteria producing efficiently acids from the

added carbohydrates (usually glucose) so that the pH value of the sausage goes down to pH 5.0 to 4.8. Moreover, the bacteria should produce acid from glucose under aerobic as well as anaerobic conditions, since conditions are comparatively anaerobic in the sausage. There were 40 strains among the remaining 285 strains, all of which produced acid from glucose under aerobic as well as anaerobic conditions at such strength that the pH value of the glucose meat broth went down below pH 5.4.

The rate of growth of the micrococci was appointed as the next selection test. This was done for the reason that the micrococcus strain added to the dry sausage mix must be able to grow rapidly in an environment with 4 to 5% NaCl content, pH 5.0 to 6.0 and a temperature of 18 to 20°C.

Accordingly, such strains were now eliminated from the residual group of 40 strains which displayed only poor growth when they had been cultivated under the said conditions during 24 hours. This left 22 strains, all of them showing strong growth in 24 hours.

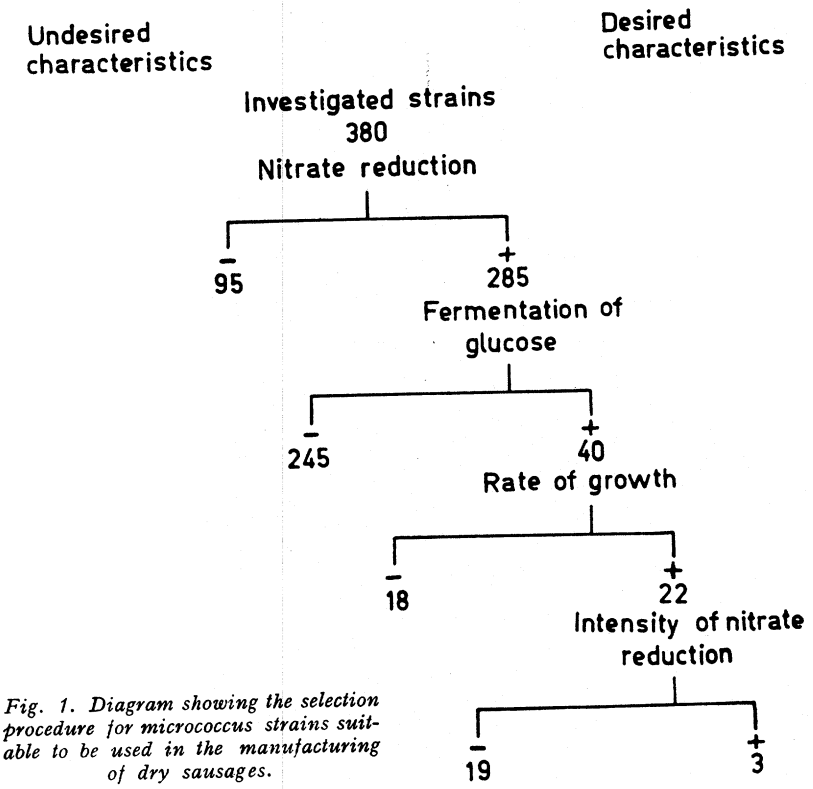


Fig. 1. Diagram showing the selection procedure for micrococcus strains suitable to be used in the manufacturing of dry sausages.

The nitrites formed by reduction of nitrates are also spontaneously further reduced into nitrogen oxide in acid environment, and qualitative demonstration of nitrite alone is not sufficient to indicate the intensity of nitrate reduction. The intensity of the nitrate reduction called forth by the said 22 micrococcus strains was therefore studied by determining the quantity of nitrates reduced by them within 24 hours.

It was found that only three of the strains reduced nitrate with such vigour that about 20 % or more of the initial amount of nitrate was reduced in 24 hours. These strains reduced nitrates also under anaerobic conditions, although the amount of nitrate undergoing reduction was then only 3 to 8 % in 24 hours.

The complete selection procedure, consisting of four tests, left thereonly three strains which turned out to possess such characteristics that they could be regarded as useful in the manufacturing of dry sausages. All three strains were non-pathogenic, coagulase-negative and non-hemolytic, and they produced lactic acid from glucose

III. Arrangement of the experimental work

1. Origin and isolation of the test strains

The following substrate was used when isolating the micrococcus strains for the present work:

Meat extract (Difco)	5.0 g
Yeast extract »	3.0 g
Peptone »	3.0 g
Glucose	1.0 g
Agar	15.0 g
Tap water.....	ad 1000 ml

The concentration of agar for slants was 20 g per litre. The reaction of the substrate was adjusted to pH 7.0. The NaCl additions were 5, 10, 15 and 20%, respectively.

The isolation of micrococci from salt brines and dry sausages was carried out as follows:

10 ml samples were taken into physiological saline from the ham and dry sausage curing brines of five factories. A sample of 8 g was taken from each one of 15 different dry sausages and homogenized (in Bühler's homogenizer) together with 72 ml physiological salt solution. Incubation at 5, 10, 25 and 37°C. The substrates had NaCl concentrations of 0, 5, 10, 15 and 20% and pH 7.0. The micrococci were transferred from a suitable dilution to slant agar substrates.

All isolated strains were subjected to the catalase test and Gram stain. All catalase-negative and Gram-negative strains were eliminated. Moreover, on microscopic examination, all rod-shaped bacteria and all bacteria occurring in distinct tetrads or packets were discarded. All aerobic and facultatively anaerobic cocci occurring individually or in irregular clusters were re-isolated into pure cultures. The primary isolates were preserved as cultures on agar slants in the refrigerator.

The number of strains isolated in this manner from brines and dry sausages is given in Table 1.

Table 1. *Number of micrococcus strains isolated from brines and dry sausages*

Incubation temperature	NaCl concentration of the substrate, and number of isolated strains					Total number of strains
	0%	5%	10%	15%	20%	
5°C	10	8	4	2	6	30
10°C	6	7	6	1	0	20
25°C	100	55	56	55	0	266
37°C	10	16	14	8	2	50
Total number of strains	126	86	80	66	8	366

233 strains were isolated from brines and 133 from dry sausage. Moreover, the following 20 strains were obtained from the type co lections of ATCC, NCTC and THLM[1]):

S. aureus	NCTC 7447	*M. ureae*	ATCC 408
S. epidermidis	NCTC 7292	*M. candidus*	ATCC 842
M. flavus	NCTC 7743	*M. cryophilus*	ATCC 122
M. agilis	NCTC 2676	*M. varians*	ATCC 399
M. aurantiacus	NCTC 1630	*M. caseolyticus*	ATCC 846
M. lysodeikticus	NCTC 2665	*M. rubens*	ATCC 186
M. luteus	NCTC 8512	*M. agilis*	ATCC 9814
M. lysodeikticus	ATCC 12698	*M. conglomeratus*	ATCC 401
M. roseus	ATCC 9815	*M. denitrificans*	THLM
M. freudenreichii	ATCC 8459	*M. halodenitrificans*	THLM

2. Preliminary study

As a first step, all micrococci isolated from brines and dry sausages (altogether 366 strains) were subjected to the following tests:

- Pigmentation of colonies
- Nitrate reduction
- Reaction in litmus milk
- Growth with $NH_4H_2PO_4$ as sole source of nitrogen
- Growth with urea as sole source of nitrogen
- Gelatine liquefaction
- Acid production from glucose, saccharose, mannitol, sorbitol and glycerol.

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[1]) ATCC = American Type Culture Collection, Washington D. C., U.S.A.
NCTC = National Collection of Type Cultures, Central Public Health Laboratory, London, England.
THLM = Technische Hogeschool Laboratorium voor Microbiologie, Delft, Holland.

The established characteristics were recorded on punched cards (Fig. 2) and could then be employed for comparison of the properties of each isolated strain with those of the other strains. Only one strain of several having identical characteristics was carried through to the continued tests. This left 171 strains out of the original 366, all of them being mutually different with regard to one or another of their characteristics. However, none of the »type strains» received from ATCC, NCTC and THLM was eliminated at this stage of the work. Including the strains from the type collections, the ultimate experimental material thus comprised 191 micrococcus strains.

3. Tests employed in the main study

All 191 strains were subjected to the following tests: Microscopic examination (Gram stain and observation of motility); catalase test; intensity of acid production from glucose under aerobic and anaerobic conditions (pH measurement after ten days' cultivation); HUGH and LEIFSON's (1953) test (cf. p. 27); indole production; nitrate reduction; ammonia production; growth with ammonium tartrate, asparagin, ammonium phosphate and urea as sole source of nitrogen; gelatine liquefaction; casein hydrolysis; reductase, peroxidase, oxidase, lipolysis, urease, hemolysis and coagulase tests; growth on potato; acid production from arabinose, xylose, mannose, galactose, fructose, maltose, lactose, saccharose, raffinose, dextrin, starch, glycogen, inulin, salicin, mannitol, sorbitol and glycerol; stab culture for establishing aerobic or anaerobic growth; starch and chitin hydrolysis; acetoin production; pigmentation of colonies; salt tolerance; reaction in litmus milk; optimum pH range, temperature range and NaCl concentration for growth.

4. Methods employed in the main study

In the investigations concerning the properties of the micrococci, inoculation was always made with a platinum wire loop from a glucose meat broth culture of the strain in question, having an age between 24 and 48 hours.

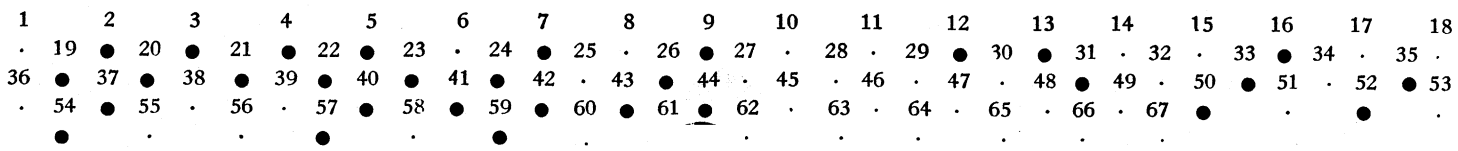
Glucose meat broth: 5.0 g meat extract, 3.0 g yeast extract, 3.0 g peptone, 1.0 g glucose and tap water ad 1000 ml.

Ammonium phosphate nutrient broth (HUCKER 1948): Nutrient broth: $NH_4H_2PO_4$ 1.0 g, KCl 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, glucose 10.0 g, 1.6 % alcohol solution

Isolation **No. 187/5**
Isolated **25. 8. 1958**

From dry sausage

1.	Nitrate reduction	−
2.	Ammonia production from peptone	++
3.	Ammonium tartrate as sole source of N	+++
4.	Asparagin » » » » »	+++
5.	Ammonium phosphate » » » » »	++
6.	Urea » » » » »	−
7.	Gelatine liquefaction	++++
8.	Casein hydrolysis	−
9.	Reductase activity	++
10.	Peroxidase »	−
11.	Oxidase »	−
12.	Lipolytic »	++
13.	Urease »	+
14.	Hemolytic »	−
15.	Coagulase »	−
16.	Growth on potato	+++
17.	Acid from arabinose	−
18.	» » xylose	−
19.	» » mannose	++++
20.	» » galactose	+
21.	» » fructose	+++
22.	» » maltose	+++
23.	» » lactose	+++
24.	» » saccharose	++++
25.	» » raffinose	−
26.	» » dextrin	++
27.	» » starch	−
28.	» » glycogen	−
29.	» » inulin	−
30.	» » salicin	−
31.	» » mannitol	+++
32.	» » sorbitol	−
33.	» » glycerol	++
34.	Aerobic	
35.	Facultatively anaerobic	+
36.	Starch hydrolysis	−
37.	Acetoin production	+
38.	Pigmentation	−
39.	Pink pigmentation	−
40.	Growth with 0 % NaCl	++++
41.	» » 5 % »	+++
42.	» » 10 % »	+++
43.	» » 15 % »	++
44.	Litmus milk test, acid production	+
45.	» » » reduction	−
46.	» » » coagulation	−
47.	» » » peptonisation	−
48.	» » » separation of whey	−
49.	Acid from glucose, aerobic, pH 7.3—6.3	
50.	» » » » , pH <6.3	+
51.	» » » anaerobic, pH 7.3—6.3	
52.	» » » » , pH <6.3	+
53.	Optimum pH range for growth 6.0—6.9	
54.	» » » » » 7.0—8.0	+
55.	» » » » » >8.0	
56.	Optimum temperature for growth 20—30°C	
57.	» » » » 31—40°C	+
58.	» » » » >40°C	
59.	Optimum NaCl conc. for growth 0 %	+
60.	» » » » » 5 %	
61.	» » » » » 10 %	
62.	» » » » » 15 %	
63.		
64.		



of bromthymol blue 1ml, and tap water ad 1000 ml. pH was adjusted to 7.6. The nutrient broth was sterilized at 105°C on three consecutive days. Incubation time 14 days.

Urea nutrient broth: The nutrient broth had the same composition as the ammonium phosphate nutrient broth, except that 1.0 g urea was added instead of $NH_4H_2PO_4$. Incubation time 14 days.

Ammonium tartrate nutrient broth: The nutrient broth had the same composition as the ammonium phosphate nutrient broth, except that 1.0 g ammonium tartrate was added instead of $NH_4H_2PO_4$. Incubation time 14 days.

Asparagin nutrient broth: The nutrient broth had the same composition as the ammonium phosphate nutrient broth, except that 1.0 g asparagin was added instead of $NH_4H_2PO_4$. Incubation time 14 days.

Catalase test: 3 ml 3 % H_2O_2 solution were added to 5 ml 24-hour-old trypsin meat broth culture. In a positive test oxygen is released immediately or within 1 to 2 minutes (HALLMANN 1953).

Indole production: 5 ml p-dimethyl-amino-benzaldehyde and subsequently 5 ml of saturated aqueous potassium persulphate solution were added to about 10 ml 3-day-old trypsin meat broth culture and the mixture was stirred. Positive reaction is indicated by red colouration (HALLMANN 1953).

Motility: A hanging drop slide was prepared from 24-hour-old meat broth culture.

Nitrate reduction: Nutrient broth: Meat extract 5.0 g, yeast extract 3.0 g, peptone 3.0 g, glucose 1.0 g, KNO_3 1.0 g, and tap water ad 1000 ml. After two days' incubation, a few drops of sulphanilic acid solution and an equal amount of α-naphthylamin were added to a sample of about 1 ml taken from the culture for observation of nitrite formation. A positive test is indicated by red colouration.

Ammonia production from peptone: Nutrient broth: Liebig's meat extract 10.0 g, Witte peptone 40.0 g, and distilled water ad 1000 ml. Ammonia production is established after incubation during 7 and 14 days with Nessler's reagent.

Gelatine liquefaction: Nutrient substrate: Meat extract 2.0 g, peptone 2.0 g, gelatine 150.0 g, and tap water ad 1000 ml. Liquefaction of the gelatine was observed after 14 days' incubation at the latest.

Casein hydrolysis (DEMETER 1943): Nutrient substrate: I) 4 % calcium caseinate solution (6.0 g casein; Merck, purum, dissolved in 150 ml 2/3-saturated $Ca(OH)_2$ water). Sterilization. II) 5.0 g peptone, 3.0 g meat extract, and 30.0 g agar dissolved in tap water ad 1000 ml. pH was adjusted to 7.5 and the solution was sterilized. Immediately prior to use 1 part of solution I and 3 parts of solution II were mixed aseptically and the mixture was poured into Petri dishes. This mixture does not tolerate sterilizing any more. Occurrence of hydrolysis produces a light halo encircling the colony; this can be strengthened by pouring acetic or hydrochloric acid on the colonies. The dishes were observed for hydrolysis during ten days.

Reductase test: Nutrient broth: Meat extract 5.0 g, yeast extract 3.0 g, peptone 3.0 g, glucose 1.0 g, and tap water ad 1000 ml. One drop of 1 % aqueous methylene blue solution was added to a 24-hour-old meat broth culture and the tubes were placed in the heating chamber at 37°C (HALLMANN 1950). They were checked for reduction of the methylene blue after 1 hour and after 4 hours. Positive reaction consists of complete discolouration, while the colour becomes green in case of slight positive reaction and no change occurs when the reaction is negative.

Oxidase test: Nutrient substrate: Meat extract 5.0 g, yeast extract 3.0 g, pepto 3.0 g, glucose 1.0 g, agar 15.0 g, and tap water ad 1000 ml. 1 % aqueous tetramethyl- phenylene-diamine hydrochloride solution was dropped upon the 2-day-old coloni (HALLMANN 1953). Positive reaction is indicated by a change to lavender colo within about one minute, and ultimately to purple. The reagent solution remai usable only a few days. It is best always to use a new solution.

Peroxidase test: The nutrient substrate had the same composition as in the oxida test. The following solutions were employed as reagents (HALLMANN 1953): I. 1 aqueous tetramethyl-p-phenylene-diamine hydrochloride solution, prepared imm diately before use; II. 3 % hydrogen peroxide solution. 9 parts of reagent I and o part of reagent II were mixed and the mixture was dropped with a pipette on th colonies of 24 to 48 hours' age. Positive reaction is indicated by pink colouration the colonies, shifting to russet or very dark red in case of strong positive reactio

Lipolysis test (JØRGENSEN 1956): Solution A: 4.0 g gelatine were dissolved in 100 distilled water at 60°C. The hot solution was adjusted with 0.1-n NaOH at pH 7 and while it was still warm, 100 ml arachis oil and 0.1 g sulphate of Nile blue we added. The mixture was emulsified in the »Starmix» emulgator for an initial peri of 5 minutes at half speed and during two minutes at full speed. A microscopic che was made to ascertain that the fat globules were no larger than 10 μ. 10 ml te tubes were dosed with 10 ml emulsion each and kept in the autoclave at 110°C for minutes. — Solution B: The following nutrient substrate was prepared: Meat extra 10.0 g, peptone 10.0 g, agar 15.0 g, and tap water ad 1000 ml. The solution w sterilized during 20 minutes at 120°C. For the substrate proper, 10 ml melte solution A and 100 ml melted solution B were aseptically mixed. The resultin solution was immediately poured into Petri dishes. Stab cultures of the organism under investigation were made on the surface of the substrate in the dishes aft it had cooled. Lipolytic organisms cause formation of free fatty acids resultin in blue colouration of the substrate around the colonies.

Urease test: Oxoid's Urea Agar Base (Code No. CM 54) was used as substrat One tablet was dissolved in 9.5 ml distilled water and the solution was sterilized durin 20 minutes at 120°C. Upon cooling to 55°C, 0.5 ml sterile 40 % urea solution we added (Oxoid, Urea 40 %, Code No. SR 20). The solution was poured into Petri dishe When the reaction is positive, the indicator turns red, owing to the ammonia liberate from urea. The reaction is complete after an incubation period of 3 to 5 hours.

Coagulase test: Bacto-Coagulase Plasma, manufactured by Difco, was used. Th contents of a 100 ml vial were dissolved in 3 ml sterile distilled water. This solutio was put in small Durham tubes, 0.5 ml in each, and two drops of a culture of 16 t 24 hours' age were added. The tubes were kept at 37°C. Most coagulase-positiv staphylococci coagulate the plasma within one hour. The strain has to be considere coagulase-negative if no coagulation occurs within three hours. Even slight coag lation has to be considered as a positive result.

Growth on potato: A raw potato was sliced as aseptically as possible and the slic were placed in test tubes. Sterilization during 30 minutes at 110°C. Incubation tim ten days, after which the appearance and colour of the growth were noted.

Hemolysis test: Blood agar dishes prepared by Lääketehdas Orion Oy were em ployed. The growth of the bacterial strains was observed in the course of incubatio after 3, 5, 7, 10 and 14 days.

Intensity of acid production from glucose under aerobic and anaerobic conditions: Nutrient broth: Meat extract 3.0 g, peptone 5.0 g, glucose 10.0 g, and tap water ad 1000 ml. For anaerobic acid production, the tubes were heated up to the boiling point immediately prior to inoculation, cooled, inoculated and sealed with Vaspar (vaseline and paraffin, 1:1). For aerobic acid production, the tubes need not be boiled. After incubation for ten days, the pH of the nutrient broth was measured, the result indicating the intensity of acid production.

Hugh and Leifson's test (1953): This test distinguishes between fermentative and oxidative bacteria. The former obtain their energy from carbohydrates by way of anaerobic fermentation, the latter by aerobic oxidation. Several oxidative bacteria produce a comparatively small amount of acid, whereas most bacteria produce alkaline metabolic products from peptone. It is therefore possible to demonstrate the acidity produced in oxidative metabolism only if a substrate poor in peptone and rich in carbohydrates is employed.

Nutrient substrate: Bacto casitone 2.0 g, NaCl 5.0 g, glucose 10.0 g, K_2HPO_4 0.3 g, agar 3.0 g, 1 % aqueous bromthymol blue solution 3 ml, and distilled water ad 1000 ml. pH adjusted to 7.1. For the test, two replicate tubes were inoculated, one of them being covered with Vaspar after inoculation. The changes in pH were followed during ten days. Fermentative organisms cause acid reaction in the open tube as well as in the tube covered with Vaspar. Oxidative organisms lower only the pH in the open tube; the reaction usually commences on the surface and gradually spreads toward the bottom.

Acid production from carbohydrates, certain glycosides, alcohols and sugar alcohols: The composition of the nutrient broth, its preparation and its sterilization were the same as in the glucose fermentation test. The following compounds were employed in the tests (10.0 g per litre of nutrient solution): arabinose, xylose, mannose, galactose, fructose, maltose, lactose, saccharose, raffinose, dextrin, inulin, starch, glycogen, salicin, glycerol, mannitol, and sorbitol. Incubation time ten days.

Stab culture: The nutrient substrate had the same compositions as in the oxidase test. After incubation during two days, the aerobic, facultatively anaerobic, microaerophilic and/or anaerobic mode of growth was noted.

Starch hydrolysis: Nutrient substrate: Meat extract 1.0 g, Bacto-trypton 5.0 g, yeast extract 1.0 g, K_2HPO_4 5.0 g, agar 20.0 g, and tap water ad 800 ml. After sterilization of the substrate, starch solution sterilized by passing through a membrane filter (50 mm dia., No. 6) and containing 1.0 g starch in 200 ml distilled water was added. After incubation during ten days, one drop of Lugol's solution was deposited on the colony. Hydrolysis of starch is indicated by a clear zone around the colony.

Acetoin production: (Voges-Proskauer test): Nutrient broth: Peptone 7.0 g (from casein), glucose 5.0 g, K_2HPO_4 5.0 g, and tap water ad 1000 ml. pH adjusted to 6.9. After incubation during three days, the presence of acetoin was observed by adding to the culture tube, per 1 ml of the culture, 0.6 ml of 5 % α-naphthol in ethanol and 0.2 ml of 40 % KOH. The solution turns red when acetoin is present.

Pigmentation of colonies: The pigmentation of the colonies was ascertained with common nutrient agar as substrate (meat extract 5.0 g, yeast extract 3.0 g, peptone 3.0 g, glucose 1.0 g, agar 15.0 g, and tap water ad 1000 ml), to which 5, 10 and 15 % NaCl, respectively, were added. Incubation at 25°C. The colour of the colonies on substrates of different salt concentration was compared 4 and 7 days after

inoculation. The following designations were used in recording the colour grey, white, yellow, green, brown, and pink.

Litmus milk test: The changes in litmus milk after inoculation were noted a 3, 7, 10 and 14 days. The following data were recorded: acid production, reducti coagulation, peptonisation and/or separation of whey.

Chitin hydrolysis (CAMPBELL 1951): Mineral substrate: K_2HPO_4 1.0 g, Mg 0.5 g, NaCl 0.5 g, $CaCl_2$ 0.1 g, agar 15.0 g, and tap water ad 1000 ml. The substr was adjusted to pH 7.0. 50.0 g chitin (H. M. Chemical Co., Ltd., Santa Moni Calif., U. S. A.) were added after having undergone the following treatment. Chi was dissolved in 50 % sulphuric acid and precipitated by dilution of this soluti with water to its 15-fold volume. The resulting finely divided chitin precipit was washed in a Büchner funnel until the filtrate was neutral (with litm paper as indicator). The wet chitin precipitate was then immediately added to t mineral base. Sterilization during 20 minutes at 120°C. The molten solution w portioned out in Petri dishes, which were inoculated with the bacterial strain un investigation by stab inoculation after they had cooled. Strains causing hydroly of chitin produce a clear zone circumscribing the colony and clearly distinguisha from the substrate (Fig. 3). The growth of the strains was recorded during the in bation time, 14 days.

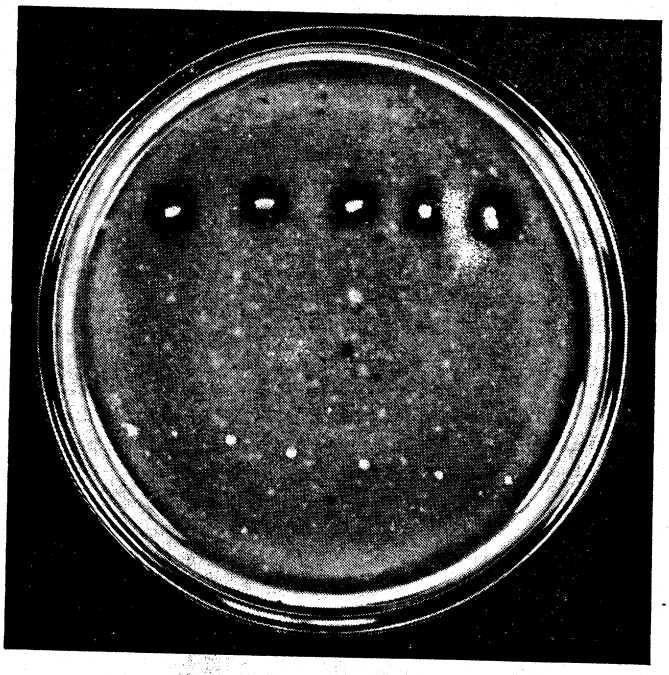


Fig. 3. Chitin hydrolysis caused by a Gram-positive rod-shaped bacterium. A clear zon has appeared around the colonies. — In the lower part of the dish, a line of colonie formed by a micrococcus strain that has caused no hydrolysis of chitin.

Salt tolerance: Agar slants were prepared by adding 0, 5, 10 and 15 % NaCl to nutrient substrate (meat extract 5.0 g, yeast extract 3.0 g, peptone 3.0 g, glucose 1.0 g, agar 15.0 g, and tap water ad 1000 ml.). All four slant substrates were inoculated with equal quantities of a 24-hour-old glucose meat broth culture of the strain to be examined. Incubation at 25°C. The extent of growth and the colour of the colonies were examined once a day, continuing up to 14 days. The optimum concentration of NaCl for growth and the salt concentration at which growth did not occur any more were estimated from the results.

Optimum temperature range for growth: Nutrient meat broth tubes (as in the preceding test omitting agar) were inoculated with 0.5 ml of the 24-hour-old glucose meat broth culture of the strain under investigation. Incubation at 0, 5, 10, 15, 20, 25, 30, 35, 40 and 45°C. After 24 hours (in some instances with slow rate of proliferation of the strain, after 48 hours) the turbidity was determined nephelometrically with the Lumetron colorimeter. The resultant D % values were plotted to show the rate of proliferation and growth as a function of temperature. For halophilic strains, the optimum NaCl concentration was used in the nutrient broth. The turbidity in several replicate tubes inoculated at the same time was followed during 14 days. From the results, the optimum temperature ranges for growth and the limits above and below which no growth occurred were determined.

Optimum pH range for growth: Nutrient meat broth: Meat extract 10.0 g, yeast extract 6.0 g, peptone 6.0 g, glucose 2.0 g, and tap water ad 1000 ml. The solution was sterilized during 20 minutes at 120°C. The following 1/7.5-m phosphate buffer solutions were prepared (SØRENSEN): (a) $Na_2HPO_4 \cdot 2H_2O$, 23.752 g/litre; (b) KH_2PO_4, 18.156 g/litre. These were sterilized by passing them through a membrane filter. Buffer solutions and meat broth were mixed in certain proportions under aseptic conditions to give the following series of pH values: pH 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0. The additions of solutions (a) and (b) were so adjusted that each final substrate contained meat broth and buffer solution in equal proportions and the nutrient solution was 1/15-molar with respect to the phosphates. The pH value of the nutrient solution was checked; if necessary, it was adjusted to the intended value with 1-n NaOH or 1-n H_2SO_4.

Each nutrient broth tube of the series was inoculated with one loopful of a 24-hour-old culture of the strain. The incubation time varied between 20 and 60 hours, depending on the particular strain. The turbidity of the broth was then measured nephelometrically with the Lumetron colorimeter. The resultant D % values were plotted to give a graph indicating the rate of growth as a function of pH. From the observations the optimum pH range for growth and the pH limits above and below which no proliferation and growth occurred were determined.

IV. General observations concerning the test strains

Only the results concerning the properties of the so-called m micrococci (that is, of the 171 isolated strains) will be treated h The properties of the 20 strains obtained from type collections not included here; these strains have only been respected in grouping of micrococci by Adansonian groups (cf. pp. 44—56).

1. Morphological and physiological characteristics

Cell morphology: Diameter 0.5 to 1.5 μ; round cells equal or sligl unequal in size. Occurring singly, or in small or large, irregular clust

Colonies on agar: Small, glossy or dull, raised; some of them wrink dry or granular, some slimy, mostly with entire edges; some colo having a darker centre; moist, round and having grey, white, yell brown, green, or pink colour.

Common characteristics: All investigated meat micrococcus str were Gram-positive and catalase-positive, aerobic or facultati anaerobic, non-motile, and they produced acid from glucose.

Other characteristics: (Table 2.) Commonest characteris displayed by the strains (present in more than 50 %) were: nit reduction, ammonia production from peptone, growth with ammon tartrate and asparagin as sole source of nitrogen, gelatine liquefact formation of reductase, growth on potato, acid production from ar nose, xylose, mannose, galactose, fructose, maltose, lactose, sac rose, dextrin and mannitol, growth in a medium containing 0 to 1 NaCl but most strongly without NaCl, acid production in lit milk, strong acid production from glucose under aerobic conditi but only slight acid production under anaerobic conditions, a optimum pH range for growth pH 7.0 to 8.0 and temperat range 20 to 30°C. Relatively common (30 to 49 %) w growth with ammonium phosphate as sole source of nitrogen, for tion of urease, acid production from raffinose, salicin, sorbitol a glycerol, formation of pigment, and optimum temperature range growth 31 to 40°C. Several micrococcus strains (20 to 29

Table 2. *Manifestation of certain physiological characteristics among 171 strains of meat micrococci.*

Characteristic	Strains displaying this characteristic	
	Number	Per cent of all strains
Nitrate reduction	143	84
Ammonia production from peptone	152	89
Growth with ammonium tartrate as sole source of N	157	92
» » asparagin » » » » »	158	92
» » ammonium phosphate » » » » »	60	35
» » urea » » » » »	33	19
Gelatine liquefaction	98	57
Casein hydrolysis	26	15
Reductase activity	98	57
Peroxidase »	14	8
Oxidase »	24	14
Lipolytic »	42	25
Urease »	78	46
Hemolytic »	0	0
Coagulase »	1	1
Growth on potato	97	57
Acid from arabinose	119	70
» » xylose	141	83
» » mannose	169	99
» » galactose	105	61
» » fructose	169	99
» » maltose	163	95
» » lactose	140	82
» » saccharose	151	88
» » raffinose	54	32
» » dextrin	139	81
» » starch	15	9
» » glycogen	6	4
» » inulin	20	12
» » salicin	56	33
» » mannitol	116	68
» » sorbitol	81	47
» » glycerol	86	50
Aerobic	19	11
Facultatively anaerobic	152	89
Starch hydrolysis	24	14
Acetoin production	9	5
Pigmentation	68	40
Pink pigmentation	2	1
Growth at NaCl concentration 0 %	166	97
» » » » 5 %	170	99
» » » » 10 %	164	96
» » » » 15 %	147	86
Litmus milk test, acid production	124	73
» » » reduction	35	21
» » » coagulation	20	12
» » » peptonisation	6	4
» » » separation of whey	7	4
Acid production from glucose, aerobic, pH 7.3—6.3	10	6
» » » » » pH <6.3	161	94
» » » » anaerobic, pH 7.3—6.3	147	86
» » » » » pH <6.3	22	13
Optimum pH range for growth 6.0—6.9	19	11
» » » » » 7.0—8.0	137	80
» » » » » > 8.0	15	9
Optimum temperature range for growth 20—30°C	117	68
» » » » » 31—40°C	53	31
» » » » » > 40°C	1	1
Optimum NaCl concentration for growth 0 %	128	75
» » » » » 5 %	41	24
» » » » » 10 %	2	1
» » » » » 15 %	0	0

caused lipolysis, reduced litmus milk and had the optimum concentration 5%. More rarely (with 1 to 19%) occu characteristics were: growth with urea as sole source of nitr formation of peroxidase, formation of oxidase, acid production starch, glycogen and inulin, starch hydrolysis, acetoin produc formation of pink pigment, coagulation, peptonisation and separ of whey in litmus milk, slight acid production from glucose u aerobic conditions and strong acid production under anaerobic c tions, optimum pH range for growth pH 6.0 to 6.9 and abov 8.0, temperature range above 40°C, and NaCl concentration None of the investigated strains produced indole from trypto nor gas from carbohydrates.

2. Acid production from carbohydrates

Most remarkable among the physiological characteristics of micrococci is their versatile ability to ferment carbohydrates. fact has also its technological significance in the manufacturi various kinds of dry meat products. This is due to the circumst that micrococci occur in large numbers, e.g., in dry sausages, to w glucose, saccharose or some other kinds of carbohydrates are a in connection with their processing in order to achieve ade production of acid. Lowering of the pH value of the sausage or the meat from about pH 6.0 to pH 5.0 is partially induced by lactic acid produced from the glycogen in the meat (lowering to a pH 5.4 to 5.5) and partially by the increased decomposition of ca hydrates into acids, caused by the bacteria. Being versatile ferme of carbohydrates, the micrococci present in the sausage mix promote the formation of acid in the products. All 171 strains stu in this work produced acid from glucose under aerobic condi and 169 of them under anaerobic conditions. More than 80% of strains moreover produced acid from xylose, mannose, fructose, mal lactose, saccharose and dextrin.

The ability of the micrococci to ferment polysaccharides into seems to be comparatively feeble. Only six of the 171 strains duced acid from glycogen and 15 strains produced acid from sta Low-molecular carbohydrates are thus found to be the most fav able sources of energy for micrococci.

Bergey's Manual (7th Edition, 1957) mentions the species *M coccus colpogenes*, which is able to utilize chitin as a source of ca as well as nitrogen. There were no strains among the investig

micrococci capable of decomposing chitin into low-molecular compounds. Only five of the 171 strains grew on mineral substrate containing chitin but no ammonium chloride.

3. Nitrogen requirements

The greater part of the present meat micrococci, more than 90% of them, were able to utilize ammonium tartrate or asparagin as their sole source of nitrogen. 35% of the strains were able to utilize ammonium phosphate, and 19% were able to utilize urea, as their sole source of nitrogen.

4. Salt tolerance

Among 366 micrococcus strains, of which 233 strains had been isolated from curing brines with high sodium chloride content (more than 12%), only five were obligatorily halophilic. The strains No. 82, 95x and 75 required about 5% NaCl for growth, and strains No. 244 and 245 about 10% (cf. Table 8, p. 50).

5. Pigmentation

Assessing the colour of a bacterial growth is always a matter of subjective observation, resulting easily in confusion when done ocularly. The right thing would be either to use a given system of colour charts (e.g. LENNING 1954; PACLT 1958), to determine by measurement the absorption maximum of the colour (STEUER 1956) or to confine the characterization to a few principal colours. The last-mentioned alternative was chosen, e.g., by SHAW et al. (1951), who employed only three colours (gold, lemon-yellow and pink) in their description of the pigmentation of growths of aerobic micrococci.

For the grouping of micrococci, however, it is most essential just to know whether the strain is pigmented and whether its growth is pink or has some other colour.

The occurrence of colour is a highly variable phenomenon, influenced by numerous factors, such as: salt concentration of the substrate, incubation temperature, age of the culture, etc. Some given compound in the substrate may have an inhibitory effect on the occurrence of colour (STEUER 1956).

In the present work, the following designations have been used in characterizing the colour of the growths of individual strains: grey,

white, yellow, green, brown, and pink. Thus, only six colours are referred to and no shades or hues are considered. In the actual process of grouping (cf. pp. 44—47) account has been taken only of whether the strain is pigmented and whether its pigment is pink.

According to this differentiation, the 171 strains of the present material show the following distribution by the colour of their colonies:

Grey	67
Brown	37
White	34
Yellow	25
Green.	6
Pink	2
Total	171 strains

Accordingly, the majority of the investigated micrococcus strains (i.e., 101 strains, or about 59 %) produced grey or white colonies, while 70 strains produced colonies of some other colour.

Table 3. *Colour of pigmentation displayed by some micrococcus strains in dependence of the NaCl content of the substrate.*

Strain No.	NaCl concentration of the substrate and colour of the growths			
	0 %	5 %	10 %	15 %
10	White	White	Grey	Grey
11	White	White	Grey	Grey
16	White	White	Grey	Grey
28	White	Brown	Brown	Brown
44	Yellow	Yellow	Grey	Grey
45	White	White	Grey	Grey
49	Grey	Yellow	Yellow	Yellow
51	White	White	Grey	Grey
53	Yellow	Yellow	Brown	Brown
67	Brown	Brown	Brown	Brown
79	White	Brown	Brown	Brown
86	Grey	Brown	Brown	Brown
89	White	Yellow	Brown	Brown
94	White	Brown	Brown	Brown
99	Grey	Grey	Grey	Brown
126	Grey	Brown	Brown	Brown
136	Grey	Brown	Brown	Brown
172	Grey	Brown	Brown	Brown
173	White	Brown	Brown	Brown
177	Grey	Brown	Brown	Brown
184	Grey	Grey	Brown	Brown
190	Yellow	Yellow	Grey	Grey
193	Grey	Yellow	Yellow	Yellow
194	Yellow	Brown	—	—
216	Yellow	Grey	Grey	Grey

In the case of some strains the colour was dependent on the sodium chloride concentration of the substrate: the colour of their colonies on substrates with 0 or 5% NaCl were white but those on substrates with 10 and 15% were clearly grey, or colonies on substrates containing 0 or 5% NaCl were yellow and colonies on substrates with 10 or 15% were grey (Table 3).

6. Acid production from glucose under aerobic and anaerobic conditions

In Bergey's Manual (7th Edition, 1957), the genera *Micrococcus* and *Staphylococcus* have been differentiated on the basis of whether the bacteria produce acid from glucose merely under aerobic conditions or under anaerobic conditions as well. However, there is no mention of how a test to this effect should be carried out nor of the method of measuring the intensity at which acid is being produced. It was found in the present investigation that nearly all meat micrococci produced acid from glucose under aerobic as well as anaerobic conditions.

It is difficult to judge the intensity of the production of acid ocularly merely by the change in colour of an indicator. On comparing the intensity of acid production occurring under aerobic and anaerobic conditions it is necessary to measure the pH of glucose meat broth culture tubes after a given incubation time and, simultaneously, that of an uninoculated control kept under identical conditions. This provides a possibility for comparing the intensity of acid production. On ocular assessment, the cultures kept in aerobic and anaerobic conditions may have the same colour, while the pH measurement reveals higher acidity in one of the two tubes.

The intensity of the acid production from glucose under aerobic as well as anaerobic conditions, caused by meat micrococci, has been tabulated in Table 4. It can be seen that 169 of the 171 strains formed acid from glucose also under anaerobic conditions. In the case of 79 of these strains the acid production was so slight that the result would necessarily have been considered negative in most instances if judged by ocular assessment only.

Table 4. *Acid production from glucose under aerobic and anaerobic conditions. Incubation time 10 days at 25°C. Altogether 171 strains.*

pH of the glucose meat broth cultures after 10 days' incubation	Strength of reaction	Number of strains displaying this action	
		Aerobic conditions	Anaerobic conditions
7,7—7,4	—	0	2
7,3—6,9	+	4	79
6,8—6,3	++	6	68
6,2—5,7	+++	31	17
below 5,7	++++	130	5

In corroboration of the above-mentioned test, also HUGH LEIFSON's (1953) test was carried out and the same results obtained although the change in colour of the indicator was equally distinct as the results of the pH measurements.

V. Attempt at grouping the test strains according to previous systems

1. Grouping by the system in Bergey's Manual, 6th Ed.

In the endeavours at grouping the 171 investigated strains of meat micrococci, the identification systems in the 6th and 7th Edition of Bergey's Manuals (1948, 1957) were compared with each other. It was evident that these principles of grouping are not as such suitable to be applied in the grouping of the micrococci occurring in meat products.

In the sixth Edition of Bergey's Manual no distinction has been made between the genera *Micrococcus* and *Staphylococcus;* all species of the kind concerned have been referred to genus *Micrococcus*. Furthermore, such anaerobic cocci have been included in this genus which have been referred to genus *Methanococcus* or *Peptococcus* in the seventh Edition. The attempt to place the present 171 strains in the species described in the 6th Edition of the Manual revealed that there were 57 strains which could not be positively referred to any description of species. Table 5 shows the placing of the present strains in the different species.

It should be noted that among 114 strains, referred to a given species in Table 5, only two strains were fully consistent with the description of the species *M. varians* given in Bergey's Manual, two with that of *M. aurantiacus* and one strain with that of *M. pyog. var. aureus*, while the others (109 strains) differed from the species descriptions in the Manual in respect of several characteristics.

For this reason, the identification of the micrococci in the present collection according to the 6th Edition of Bergey's Manual seems to remain rather unsatisfactory.

In identifying the strains in accordance with the 6th Edition of Bergey's Manual, it was necessary to place in the species *M. luteus*, *M. flavus*, *M. conglomeratus* and *M. varians* such strains which show yellow, green or brown pigmentation. The key in the said Manual

Table 5. *The placing of 171 strains of meat micrococci in the species described Bergey's Manual, 6th Edition.*

Name of species	Number of strains
M. luteus	10
M. ureae	1
M. freudenreichii	7
M. flavus	3
M. candidus	7
M. conglomeratus	11
M. varians	9
M. caseolyticus	—
M. pyog. var. aureus	7
M. pyog. var. albus	31
M. citreus	—
M. aurantiacus	18
M. epidermidis	10
M. roseus	—
M. cinnabareus	—
M. rubens	—
M. rhodochrous	—
M. agilis	—
M. morrhuae	—
Unspecifiable	57

contains the characterizations »Yellow pigment on agar media« »No pigment produced«. In the interests of grouping it would rat suffice to specify »Pigment produced on agar media« and »No pigm produced«.

2. Grouping by the system in Bergey's Manual, 7th Ed.

In the seventh Edition of Bergey's Manual, the Gram-posi catalase-positive cocci have been referred to genera *Micrococcus* *Staphylococcus*. These genera are distinguished by the ability of t- bacteria to produce acid from glucose in aerobic or also in anaer conditions. Thus, cocci producing acid under aerobic conditions would belong to genus *Micrococcus*, while those having this ability under anaerobic conditions would belong to genus *Staphylococcus*. further subgrouping of the latter genus rests on the coagulase and on the production of acid from mannitol.

If the present 171 meat micrococcus strains (i.e., all strains cerned in this work except those obtained from type collecti- would be grouped on the basis of their ability to produce acid f glucose under aerobic conditions alone or under anaerobic condit as well, 169 of these strains would have to be referred to g

Staphylococcus and only two of them to genus *Micrococcus* (cf. Table 2, p. 31). Only one of the 169 strains was coagulase-positive, and it would thus have to be identified as *Staphylococcus aureus*. Among the remaining 168 strains, those producing acid from mannitol, i.e., 116 strains, would be given the name *S. epidermidis*. However, the strains differed from *S. epidermidis* in respect of several characteristics and they cannot be considered as representing the said species. The other 52 strains would also belong to genus *Staphylococcus* but they would not be representatives of any named species.

For these reasons the grouping of the present collection of cocci in the genera *Micrococcus* and *Staphylococcus* and closer identification of the individual strains in accordance with the 7th Edition of Bergey's Manual was not regarded to be advisable.

3. Comments on the systems in Bergey's Manuals

The treatment of the matter in the 6th Edition of Bergey's Manual calls forth, among others, the following comments: The species *M. pyogenes var. aureus* (orange pigment) and *M. citreus* (yellow pigment) cannot be differentiated merely on the basis of the colour of their growths. The species *M. pyogenes var. albus*, *M. pyogenes var. aureus* and *M. citreus* are so closely similar with respect to their characteristics that they cannot be mutually distinguished by the stated characteristics. The sole differentiating characteristic might, perhaps, be their reaction in the coagulase test.

According to most recent research, the species *M. cinnabareus* and *M. rhodochrous* belong to genus *Mycococcus* of the family *Mycobacteriaceae* and therefore cannot be considered as micrococci.

In the 7th Edition of Bergey's Manual the species *M. aurantiacus* is missing, and it has not been referred to any other genus or family.

4. Grouping by the system of SHAW et al.

Treatment of the present material in accordance with the system presented by SHAW et al. (1951) resulted in the following distribution of the 171 strains by species of the genus *Staphylococcus:*

S. aureus	1
S. saprophyticus	9
S. lactis	159
S. roseus	2
S. afermentans	0
Total	171 strains

This indicates that the meat micrococci constitute a grou their own, presenting characteristics that occur with *S. lactis* of S et al. Also KITCHELL (1958) has observed that the majority of micrococci from meat and brines investigated by him find their pl in the *S. lactis* group of SHAW et al.

According to HILL's (1959) investigations, however, the *S. lactis* group (and also the *S. afermentans* group) shows little sistency in its composition, which seems to be highly heterogen Rather little information concerning the characteristics of micrococci and their possible similarities and dissimilarities with strains is therefore conveyed by the fact that they would belo the group *S. lactis* as defined by SHAW et al.

VI. The Adansonian classification of the test strains

It has just been reported that the meat micrococci concerned in this work could not successfully be divided into groups and the individual strains specified as representing certain species by applying the grouping systems given in Bergey's Manuals (1948, 1957) or those presented by SHAW et al. (1951) and by HILL (1959). This suggested the inference that the tests constituting the criteria for the said systems of grouping are such that they fail in differentiating adequately between the meat micrococci. As a corollary, the thought was called into life that the grouping of these bacteria should obviously be based on a set of tests other than those specified in the said grouping systems.

It would now have been entirely arbitrary to appoint some specific characteristic, or several characteristics, to serve as a criterion for the further subgrouping of the strains, and the author therefore decided to apply to the present material the so-called Adansonian principle of classification developed by SNEATH (1957 a, b). To the author's knowledge, this discipline has so far been used in only few instances as a tool in aid of the classification of bacteria. It is therefore thought to be appropriate to outline here, with reference to SNEATH's (1957 b) paper, the foundation on which this procedure rests and how the division of bacteria into so-called Adansonian groups is carried out.

1. Description and example of the grouping procedure

SNEATH (1957a, b) has put forward a new idea on the principles by which bacteria should be classified. The method suggested by him is ultimately based on the opinion expressed in 1763 by MICHEL ADANSON that each characteristic should be treated as equal in value. In deference to ADANSON, SNEATH calls his method for the classifying of bacteria the »Adansonian classification». It should be realized that this method is radically different from the procedure generally employed in classifying bacteria, by which the classification is done on the basis of characteristics chosen in a relatively arbitrary manner.

In any application of SNEATH's method, all characteristics of

the investigated strains are given equal weight and all examined characteristics of all strains are mutually compared. It is then possible to note the number of similar and dissimilar features (n_s and n_d, respectively) and to compute from them the similarity, S (S, in $\% = \frac{n_s}{n_s + n_d} \times 100$). The sum $n_s + n_d$ will obviously be the total number of characteristics under consideration.

In the method of analysis used in the present work, only positive features are employed. No account is taken of negative features, that is, features which are absent in all objects of the study in hand. This is reasonable since the class of absent features is almost infinite and there is no way of making any logical choice of them. Although this means that some negative data are not analyzed, it does not reduce the amount of information contained in the classification.

Numerous tests have to be carried out with each individual strain for their mutual comparison and for computation of the S values. In the work presented here, for instance, 62 tests were performed with each one of 191 strains (171 strains of meat micrococci plus 20 strains from type collections). In stating the results of the tests by means of the so-called i × m (strains × features) table, care has to be taken not to state one and the same fact twice, in different form. For instance, the statement »Growth occurs at 37°C» is redundant if the statement »Growth occurs at temperatures between 12 and 44°C» has been made in another connection.

In order to render the substance of the Adansonian classification readily understandable, part of the present material, consisting of the three strains No. 245, 238 and 24 (cf. Fig. 6, p. 55, and Table 13, p. 67) shall be treated here as an example. The following symbols will be used:

- i = number of strains of bacteria
- m = number of positive features which are possessed by at least one strain
- n_s = number of similar features possessed by both strains of a pair. It does not include the features which are not possessed by either strain.
- n_d = number of dissimilar features, that is, the number of positive features possessed by the first strain but not by the second plus the number possessed by the second strain but not by the first.
- $n_f = n_s + n_d$
- S = Similarity = n_s/n_f
- D = Dissimilarity = n_d/n_f

The S and D values may be expressed as decimal fractions or as percentages. The calculation of the S value is carried out on the basis of the results in the i × m (strains × features) table as follows:

i × m table

Strain No.	Growth with 0% NaCl	Growth with 15% NaCl	Acid production from glucose
245	—	+	+
238	+	+	+
24	+	—	+

In comparing any two strains, one starts by noting the number of those tests in which a positive result was obtained with both strains. It is seen that two tests (growth with 0% NaCl; acid production from glucose) were positive for strain No. 238 as well as strain No. 24. Strains No. 245 and 24 had a positive result in common in only one test (acid production from glucose).

These comparisons are made for all possible ways of pairing the strains and the resulting table of n_s values is as follows. Note that where both strains are negative, this is not included in finding n_s.

n_s table

Strain No.	245	238	24
245	2	2	1
238	2	3	2
24	1	2	2

Next, the number of different features for each pair of strains is calculated, that is, those for which one strain has produced a positive, the other strain a negative result. This gives the following table of n_d values:

n_d table

Strain No.	245	238	24
245	0	1	2
238	1	0	1
24	2	1	0

The value of $n_s/(n_s + n_d)$ can then be obtained for each way of pairing the strains, giving the table of S values:

S table

Strain No.	245	238	24
245	2/2	2/3	1/3
238	2/3	3/3	2/3
24	1/3	2/3	2/2

The S values can be stated as percentages, as has been done in the following version of the i × i table:

i × i table

Strain No.	245	238	24
245	100.0	66.7	33.3
238	66.7	100.0	66.7
24	33.3	66.7	100.0

Part of the tables of n_s, n_d and S is redundant and may be omitted, since it is obvious that any strain when compared with itself will have S = 100 % while the upper right-hand part of the table is a mirror image of the lower left-hand part. Of course no useful results can be expected from considering only three tests, but the same method is useful for larger numbers of features.

In classifying bacteria in the manner described above, use is mostly made of an electronic computer, which performs the computation of the similar and dissimilar features from the i × m (strains × features) table and joins the strains of bacteria into groups on the basis of their S values. Compiled to form an i × i (strains × strains) table, these data show the S value between different groups. In the present instance no electronic computer was available and this work was done entirely by hand, although an expenditure of several months' computing time was necessary in order to find all S values in this manner. It may be mentioned that HILL's (1959) investigations concerned only a material of 49 strains, for which he determined a classification by Adansonian groups with the aid of an electronic computer.

2. S values for the test strains

For computation of the S values, the results of all tests enumerated in the foregoing with each of the 191 strains were mutually compared, excepting only the Gram reaction, motility and catalase and indole tests. These were excluded because all investigated strains displayed

the same behaviour in them. Moreover, HUGH and LEIFSON's (1953) test was not taken into account because, instead, the pH value in the glucose meat broth tubes was measured after a given time of incubation. There were altogether 62 characteristics occurring as points of comparison, and the sum $n_s + n_d$ varied between 30 and 45 in the different instances. The grouping was carried out with the aim in mind that a strain of micrococci referred to a given group should have S not less than 60% with respect to any other strain of this group. However, some exceptions from this rule had to be made in the Groups I, II and III set forth in Table 8, p. 50. As no electronic computer was available for computation of the similar and dissimilar features and for the grouping work proper, the work was arranged to take place in the following manner.

The i × m (strains × features) table introduced by SNEATH (1957 b) (cf. p. 43) was compiled, its columns representing the different characteristics and the rows representing the strains (cf. Fig. 4). A positive feature was scored by entering a cross in the proper square, while the square was left empty for negative features.

The number of similar features (n_s) and dissimilar features (n_d) of each pair of strains was then determined in the following manner. As a first step, the number of similar and dissimilar features of the strains, occupying the first and second row in the table was found. The top row of the i × m table, stating the properties of the first strain, was then detached with the scissors and tacked to the working desk so that the rest of the table could be passed through under this row. When this was done, each one of the remaining 190 strains could be compared with the first strain by placing their respective rows in juxtaposition and the number of similar and dissimilar features could be determined. Next, the row now occurring topmost in the truncated table was detached and the procedure was repeated, 189 strains being compared with that separated from the table. Continuing in the same manner, all required n_s and n_d values were found after a total of $\frac{191 \times 190}{2} = 18{,}145$ comparisons[1]).

[1]) It should be noted that this figure only gives the number of comparisons between pairs of rows in the table, i.e., the total number of comparisons with respect to one particular characteristic of the strains. There were altogether 62 investigated characteristics, and the average number of similarities between strains (n_s) was 30 and that of dissimilarities (n_d) was 30. In comparing the strains in pairs and computing the n_s and n_d values for the pairs, a total of $18{,}145 \times 30 + 18{,}145 \times 30 = 1{,}088{,}700$ individual comparisons had thus to be made. It has been an extremely time-consuming job to carry through a programme of this extent without recourse to an electronic computer. In fact, two persons were intensively occupied with this work during more than two months.

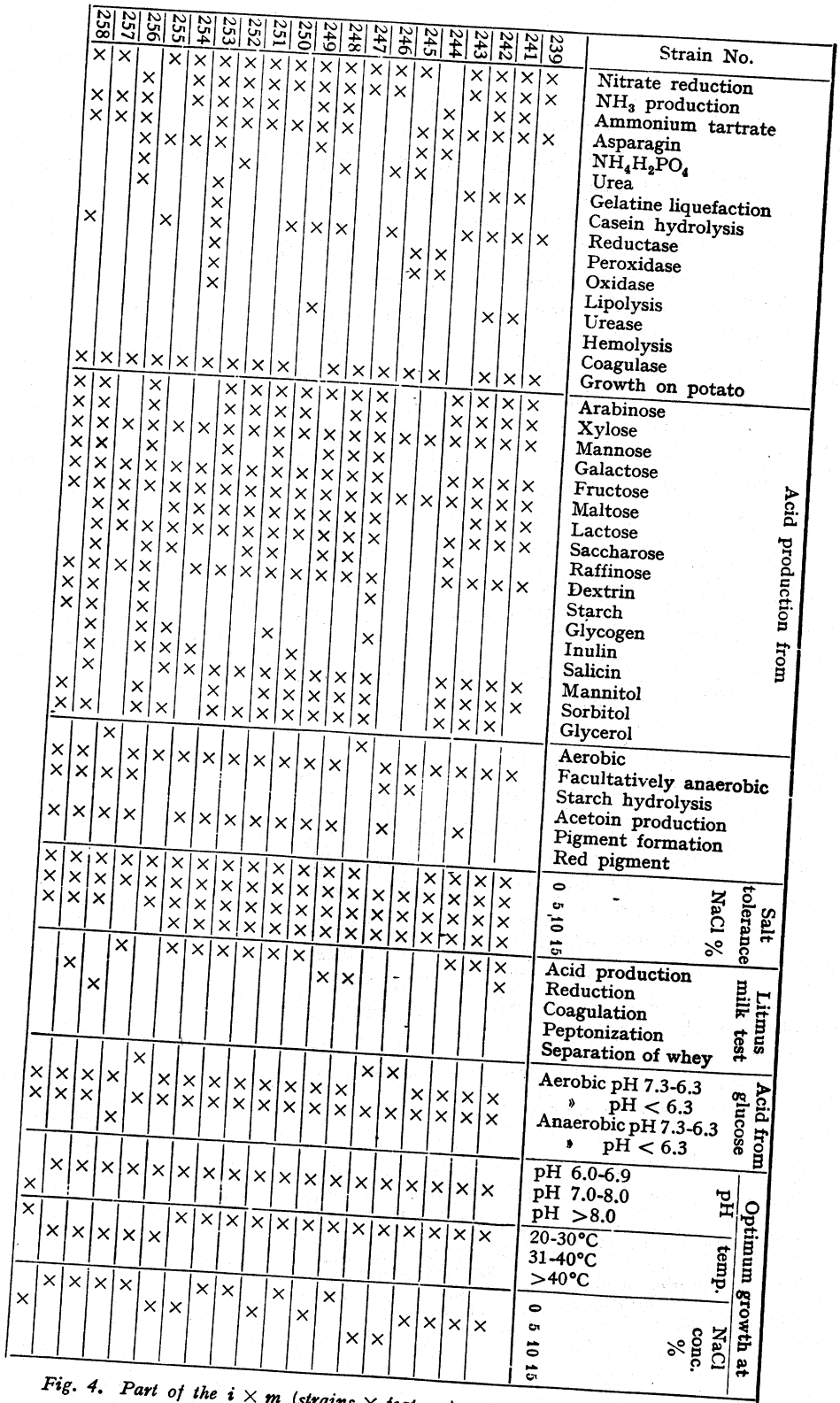


Fig. 4. Part of the i × m (strains × features) table for the meat micrococci.

The necessity of computing the S value by the formula $S\% = \frac{n_s}{n_s + n_d} \times 100$ separately for each individual comparison was obviated by compiling a table giving S, in %, for different combinations of integer values n_s and n_d, the $n_s + n_d$ (similarity × dissimilarity) table (Table 7). The table reproduced here carries the highest n_s and n_d value 30, but some instances occurred, in which n_s or n_d was between 35 and 40.

3. Grouping of the test strains

It was a difficult and cumbersome work to arrange an experimental material of this extent (191 strains; 18,145 S% values) by Adansonian groups without an electronic computer. It was done as follows: Of all S% values computed for each strain with respect to each other strain, those in the range of 80—100% were sorted out. The corresponding pairs of strains constituted a number of basic groups. One after the other, the remaining strains were then tried for their fit into each one of these groups; they were referred to the group in which they were found to have the highest set of S values with the other members of the group. Table 6 will serve as an illustration of the procedure.

Table 6. *Grouping of the strains of meat micrococci on the basis of the S values.*

Strain No.	Group I				Group II		Group III			Group IV			Group V	
	241	242	238	**22**	228	234	26	31	**15**	52	53	101	162	174
22	79	76	82	*100*	69	71	71	65		69	65	68	75	75
15	69	67	71	71	74	76	76	81	*100*	74	69	72	60	60

For instance, it can be seen from Table 6 that strain No. 22 belongs to Group I, not to Groups II to V, on the basis of the S% values. In other words, higher S% values are obtained for strain No. 22 with respect to the strains in Group I than with respect to those in Groups II to V. Similarly, strain No. 15 belongs to Group III, not to Groups I to II or IV to V. When a strain has been referred to a given group, it is also written among the strains constituting this group. Thus, in Table 6, strain No. 22 has been entered in Group I, but not strain No. 15. This strain has been written in among the strains of Group III.

Table 7. *S % values for different combinations of n_s and n_d ($n_s \times n_d$ table prepared for determination of the S values). n_s = number of similar features, n_d = number of dissimilar features.*

$n_d \rightarrow$	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
n_s 5	50	45	42	38	36	33	31	29	28	26	25	24	23	22	21	20	19	19	18	17	17	16	16	15	15	14	5
↓ 6	55	50	46	43	40	38	35	33	32	30	29	27	26	25	24	23	22	21	21	20	19	19	18	18	17	17	6
7	58	54	50	47	44	41	39	37	35	33	32	30	29	28	27	26	25	24	23	23	22	21	21	20	19	19	7
8	62	57	53	50	47	44	42	40	38	36	35	33	32	31	30	29	28	27	26	25	24	24	23	22	22	21	8
9	64	60	56	53	50	47	45	43	41	39	38	36	35	33	32	31	30	29	28	27	26	26	25	24	24	23	9
10	67	63	59	56	53	50	48	45	43	42	40	38	37	36	34	33	32	31	30	29	29	28	27	26	26	25	10
11	69	65	61	58	55	52	50	48	46	44	42	41	39	38	37	36	34	33	32	31	31	30	29	28	28	27	11
12	71	67	63	60	57	55	52	50	48	46	44	43	41	40	39	38	36	35	34	33	32	32	31	30	29	29	12
13	72	68	65	62	59	57	54	52	50	48	46	45	43	42	41	39	38	37	36	35	34	33	33	32	31	30	13
14	74	70	67	64	61	58	56	54	52	50	48	47	45	44	42	41	40	39	38	37	36	35	34	33	33	32	14
15	75	71	68	65	63	60	58	56	54	52	50	48	47	45	44	43	42	41	39	38	38	37	36	35	34	33	15
16	76	73	70	67	64	62	59	57	55	53	52	50	48	47	46	44	43	42	41	40	39	38	37	36	36	35	16
17	77	74	71	68	65	63	61	59	57	55	53	52	50	49	47	46	45	44	43	41	40	40	39	38	37	36	17
18	78	75	72	69	67	64	62	60	58	56	55	53	51	50	49	47	46	45	44	43	42	41	40	39	38	38	18
19	79	76	73	70	68	66	63	61	59	58	56	54	53	51	50	49	48	46	45	44	43	42	41	40	40	39	19
20	80	77	74	71	69	67	65	63	61	59	57	56	54	53	51	50	49	48	47	45	44	43	43	42	41	40	20
21	81	78	75	72	70	68	66	64	62	60	58	57	55	54	53	51	50	49	48	47	46	45	44	43	42	41	21
22	81	79	76	73	71	69	67	65	63	61	59	58	56	55	54	52	51	50	49	48	47	46	45	44	43	42	22
23	82	79	77	74	72	70	68	66	64	62	61	59	58	56	55	53	52	51	50	49	48	47	46	45	44	43	23
24	83	80	77	75	73	71	69	67	65	63	62	60	59	57	56	55	53	52	51	50	49	48	47	46	45	44	24
25	83	81	78	76	74	71	69	68	66	64	63	61	60	58	57	56	54	53	53	51	50	49	48	47	46	45	25
26	84	81	79	76	74	72	70	68	67	65	63	62	60	59	58	57	55	54	53	52	51	50	49	48	47	46	26
27	84	82	79	77	75	73	71	69	68	66	64	63	61	60	59	57	56	55	54	53	52	51	50	49	48	47	27
28	85	82	80	78	76	74	72	70	68	67	65	64	62	61	60	58	57	56	55	54	53	52	51	50	49	48	28
29	85	83	81	78	76	74	73	71	69	67	66	65	63	62	60	59	58	57	56	55	54	53	52	51	50	49	29
30	86	83	81	79	77	75	73	71	70	68	67	65	64	63	61	60	59	58	57	56	55	54	53	52	51	50	30
	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	

In this manner, at first 20 basic groups were made up of the 171 strains of meat micrococci, the various pairings of strains within one group obtaining S % values between 80 and 100. Altogether 52 strains found their places in these groups. 89 of the other 119 strains could be placed in these groups, but the 30 remaining strains could not be referred to them, nor did they form any groups among themselves. In the following these strains will be referred to as »solitary strains».

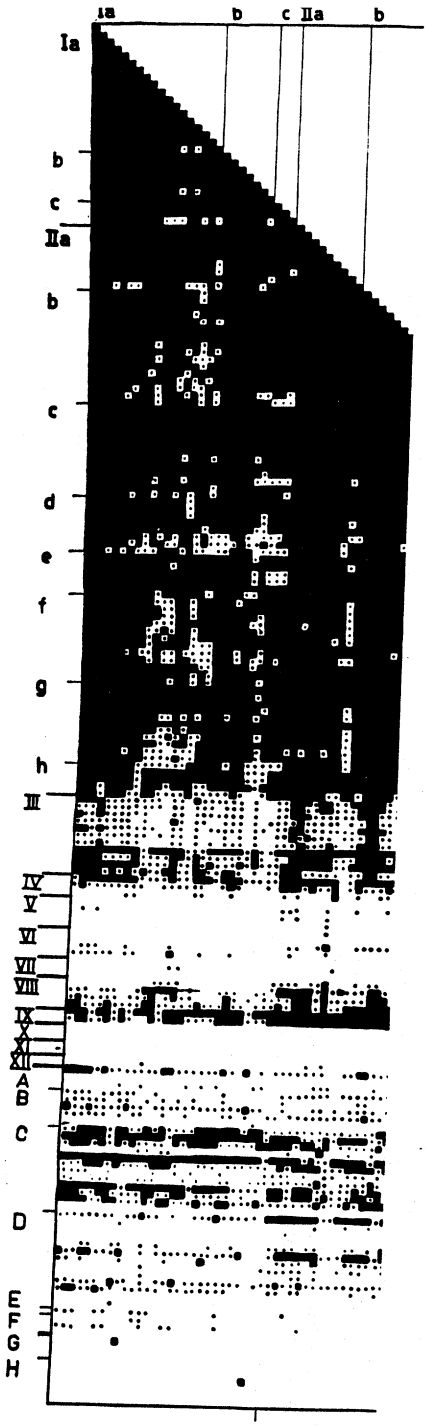
As a consequence of the principle followed in the grouping procedure, that a strain belonging to a given group should produce an S % value between 60 and 100 with any other strain of the same group, several groups had to be lumped together. In the ultimate division into groups, the Groups I to X (Table 8) were thus made up of the meat micrococci investigated in this work. The solitary strains have also been gathered into groups A to H in Table 8 for the reason that they will later be found to take their places in the Groups I to XII in Fig. 6 p. 55. This was done in order to avoid the loss of perspicuity which Fig. 6 would have suffered if the solitary strains had been entered individually in the scheme.

It can be seen from Table 8 that 141 of the 171 strains have found their places in the Groups I to X formed by these meat micrococci. Groups IX and X contain only two strains each. 30 strains of meat micrococci did not form any groups among themselves, nor did they find a place in any of the Groups I to X. The so-called type strains formed only the Groups XI and XII, only two strains finding their place in each; but these, as well as Groups IX and X, have not been considered as particular groups in this connection. The other 16 type strains could not be referred to any of the Groups I to XII. The majority of the meat micrococci (108 strains) found their places in Groups I and II, which could be further subgrouped, although the groups formed in this way could not be differentiated by means of any of the investigated characteristics. Groups I and II were mutually closely similar but they differed clearly from Groups III to XII (cf. also Fig. 5).

A clear idea of the grouping of the meat micrococci is given by the i × i (strains × strains) table (cf. p. 44), which was prepared from the data described above. This table contains the S % values for all possible pairs of strains and it was rearranged with respect to the succession of the strains so that groups clearly distinguishable from each other by the S % values have been formed. This rearranged i × i table is also represented by the graph in Fig. 5, where three different symbols stand for three ranges of S % values. This

Table 8. *Grouping of the micrococci on the basis of the S% values, and placing of the groups in the system presented by* SHAW *et al.*

Group by the i × i (strains × strains) table		Number of strains	Isolation numbers of the strains	Species designated by the system of SHAW et al.	Species designated by the author's system (cf. pp. 57–65).
	I a	18	241, 242, 238, 131, 168, 252, 239, 89, 169, 232, 32, 90, 243, 126, 143, 247, 22, 78	*S. lactis*	Halotolerant, subgroup **c**
	b	7	162, 174, 127, 249, 141, 71, 145	*S. lactis*	
Total 28	c	3	137, 150, 136	*S. lactis*	
	II a	9	228, 234, 85, 235, 237, 155, 212, 103, 224	*S. lactis*	Halotolerant, subgroup **d**
	b	16	33, 73, 80, 151, 138, 28, 77, 72, 25, 10, 100, 117, 108, 110, 30, 16	*S. lactis*	
	c	13	51, 60, 165, 222, 170, 45, 26, 31, 146, 157, 15, 124, 123	*S. lactis*	
	d	8	52, 101, 53, 79, 220, 67, 190, 223	*S. lactis*	
	e	6	102, 178, 152, 163, 250, 227	*S. lactis*	
	f	12	184, 189, 204, 177, 181, 27, 58, 95, 203, 176, 11, 180	*S. lactis*	
	g	12	94, 251, 99, 149, 216, 207, 208, 13, 217, 196, 134, 49	*S. lactis*	
Total 80	h	4	86, 248, 173, 233	*S. lactis*	
	III	11	187, 214, 210, 215, 47,	*S. saprohyticus*	*S. saprophyticus*
			191, 104, 199, 179, 201, 44	*S. lactis*	Halotolerant, subgroup **c**
	IV	3	21, 41, 62	*S. lactis*	subgroup **e**
	V	4	24, 23, 43, 188	*S. lactis*	Non-halotolerant, subgroup **i**
	VI	4	219, 119, 118, 185	*S. lactis*	subgroup **l**
	VII	3	111, 256, 115	*S. lactis*	subgroup **i**
	VIII	4	192, 205, 230, 195	*S. lactis*	Halotolerant, subgroup **f**
	IX	2	166, 172	*S. lactis*	subgroup **d**
	X	2	244, 245	*S. lactis*	Halophilic, subgroup **b**
	XI	2	ATCC 399, 401	*S. lactis*	Non-halotolerant, subgroup **k**
	XII	2	ATCC 8459, 8425	*S. saprophyticus*	*S. saprophyticus*
Solitary strains	A	3	193, NCTC 7447, ATCC 8460	*S. aureus*	*S. aureus*
	B	4	211, 200, 14, 198, NCTC 7292	*S. saprophyticus*	*S. saprophyticus*
	C_1	6	74, 92, 128, 144, 221, 253	*S. lactis*	Halotolerant, subgroup **c**
Total:	C_2	3	153, 202, 246	*S. lactis*	subgroup **d**
30 meat	C_3	3	109, 194, NCTC 1630	*S. lactis*	subgroup **e**
micro-	D_1	4	7, 39, 186, NCTC 2665	*S. lactis*	Non-halotolerant, subgroup **i**
cocci;	D_2	6	3, 5, 254, 255, 257, 258	*S. lactis*	subgroup **k**
16 type	D_3	3	4, 218, ATCC 408	*S. lactis*	subgroup **l**
strains	E	1	82	*S. lactis*	Halophilic, subgroup **b**
	F	2	75, 95x	*S. roseus*	subgroup **a**
	G	4	NCTC 2676, ATCC 9815, 186, 9814	*S. roseus*	*M. roseus*, non-halotolerant, subgroup **h**
	H	6	NCTC 7743, 8512, ATCC 12698, 12226, *M. denitrificans*, *M. halodenitrificans*	*S. afermentans*	Non-fermentative micrococci


Ia
b
c
IIa
b
Ia
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IIa
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III
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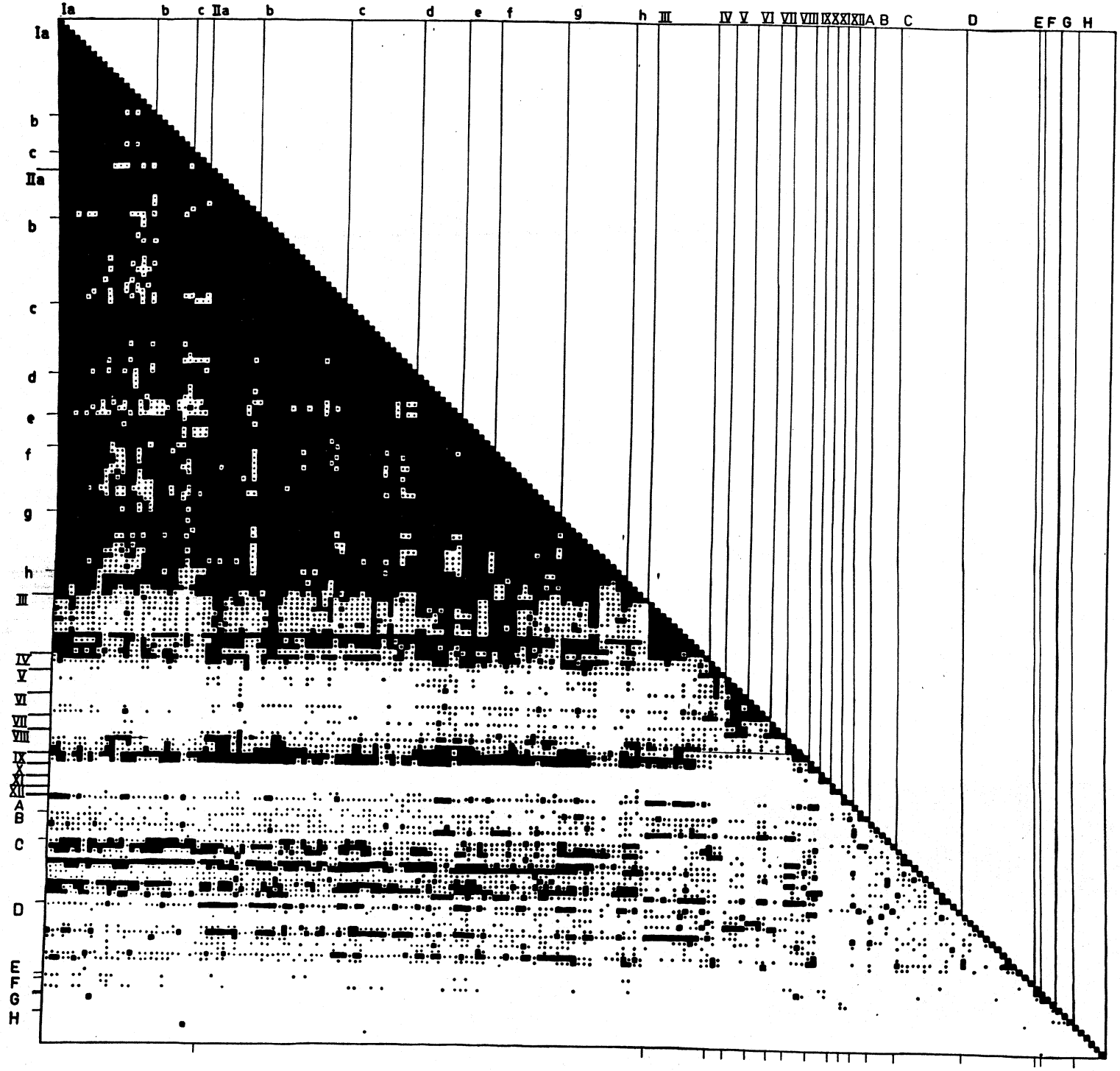

Fig. 5. Diagrammatic representation of the full S % value table.
Legend: ■ S % = 60–100 %, ▨ S % = 30–59 %, □ S % = 0–29 %.

coarse gradation was deliberately chosen as the only means to obtain a concise and lucid picture of the grouping. The numerical i × i table with its data of no less than 191 strains is not satisfactory in this respect, in addition to which it is too unwieldy for reproduction here (original size about 100 cm × 100 cm).

Fig. 5 reveals that Groups I and II are clearly distinct from Groups III to XII. It is to be noted that none of the so-called type species, i.e., the strains from the NCTC, ATCC and THLM (cf. p. 22) collections, have found their place in any of the Groups I to X. This implies that there was not one strain among the 171 micrococcus strains derived from meat products which would have been consistent in respect of its characteristics with some of the type species in the collections of NCTC, ATCC and THLM. Thus, the author feels justified in stating that the micrococci occurring in meat products form their own particular group, which seems to be well-deserved of closer study from a taxonomic point of view.

Scrutiny of the identification of the groups of meat micrococci presented in Table 8 (p. 50) by the system of SHAW et al. reveals that Groups I, II and IV to X in their entirety represent the *Staphylococcus lactis* group. Of the strains in Group III, five fell into the *S. saprophyticus* group and six into the *S. lactis* group (cf. Table 8). None of the actual groups became referred to the groups *S. aureus*, *S. roseus* and *S. afermentans* of SHAW et al. Among the solitary strains of meat micrococci, 23 found their place in the *S. lactis* group, one in the *S. aureus*, two in the *S. roseus* and four in the *S. saprophyticus* group.

The results obtained by Adansonian grouping of the meat micrococci show that most meat micrococci represent the group *S. lactis* of SHAW et al. and that this group can be further subgrouped (cf. p. 39).

4. The grouping system for micrococci

The usual procedure in grouping a great number of different strains of bacteria is to choose, on the basis of experience or rather arbitrarily, some given characteristic for the basis of the grouping. According to whether this characteristic is present or absent (positive or negative feature), the bacteria are divided into two or more groups. The subgrouping of genus *Micrococcus* in Bergey's Manuals (1948, 1957) is based on the nitrate-reducing capacity, pigmentation, fermentative reactions, reaction in litmus milk, liquefaction of gelatine,

etc. However, these characteristics are not thought to carry any demonstrably greater significance than others and there should be no well-motivated reason for the grouping of micrococci on this particular basis.

On the other hand a natural system of classification is obtained, as SNEATH (1957 a, b) has shown, if a sufficient number of characteristics of a considerable number of strains is taken into account and all characteristics are considered equal in value, comparing all strains mutually with respect to all characteristics. After this has been done, the groups formed in this manner can be mutually compared with regard to their salient characteristics. It is then possible to establish whether there are such features which occur in one group but not in the other. For instance, if in Group »a» all strains reduce nitrate to nitrite but none of the strains in Group »b» has this property, then one may choose this characteristic to be used as a characteristic differentiating between the groups.

It was found in the case of the present material that, among the characteristics mentioned in the foregoing (cf. table 2, p. 31), only the ability of the bacteria to grow in media containing 0% and 15% NaCl, respectively, were characteristics by which the meat micrococci (in particular the strains representing the group *S. lactis* of SHAW et al., i.e., those belonging to HILL's group of »fermentative miscellaneous staphylococci») could be further subdivided into three groups. The groups of micrococci coming into existence through this grouping have been named in the present investigation according to their salt tolerance, that is, they are referred to as »halotolerant, non-halotolerant and halophilic fermentative micrococci» (Table 9).

Table 9. *Grouping of the meat micrococci by their salt tolerance.*

Groups of the Adansonian classification	Growth in media with		Suggested appellation
	0%-NaCl	15% NaCl	
I, II, III[1]), IV, VIII, IX	+	+	Halotolerant fermentative micrococci
V, VI, VII	+	—	Non-halotolerant fermentative micrococci
X	—	+	Halophilic fermentative micrococci

In the present work HILL's (1959) suggestion has been adopted that the pigment-forming, non-fermentative strains should be separated

[1]) The strains of *S. lactis* type in Group III.

from the staphylococci and placed in another genus (*Micrococcus*). Moreover, the *S. lactis* group of SHAW et al. (HILL's »fermentative miscellaneous staphylococci«) has been referred to the micrococci. The coagulase-positive strains (SHAW et al.: *S. aureus*) and the acetoin-positive strains (SHAW et al.: *S. saprophyticus*) will then be labelled as staphylococci and all other strains as micrococci (cf. Fig. 6). But in the system presented by HILL (cf. p. 13) the strains

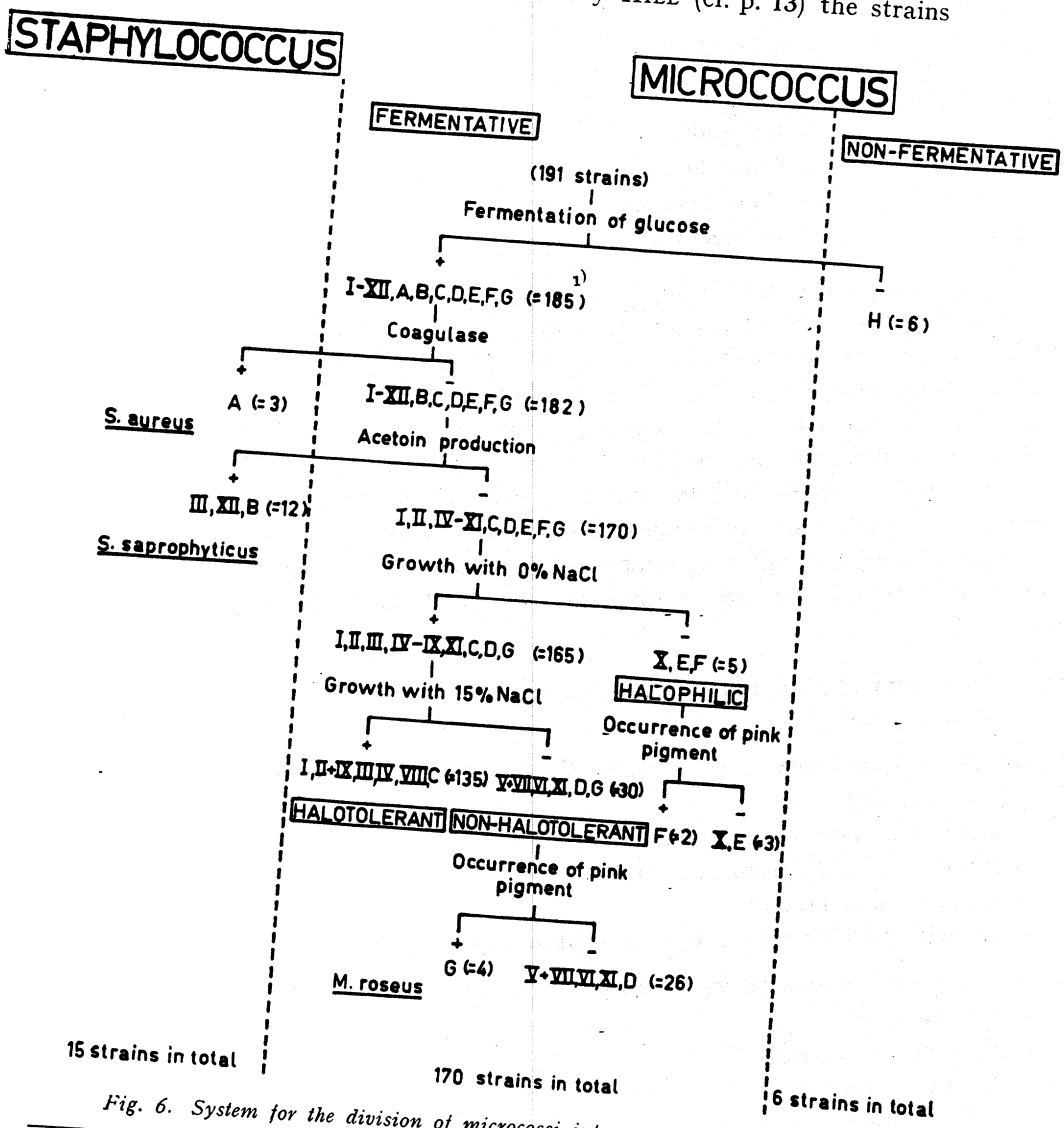

Fig. 6. System for the division of micrococci into groups (cf. Table 8, p. 50).

1) The figures within brackets indicate the number of strains.

forming pink pigment do not belong to the group of »fermentative miscellaneous micrococci»; *M. roseus*, though fermentative, would therefore be excluded from this group. For this reason also strains forming pink pigment have been considered fermentative in the present investigation. The following division of the micrococci into fermentative and non-fermentative micrococci is then obtained (see also Fig. 6):

Staphylococcus: *S. aureus*
S. saprophyticus

Micrococcus: Fermentative:
1. Halophilic
2. Halotolerant
3. Non-halotolerant

Non-fermentative

The meat micrococci will thus belong to the group of »fermentative micrococci». The system for their grouping presented by HILL (1959) can then be supplemented as shown in Fig. 6 (cf. also p. 13).

The groups indicated in Fig. 6 by the letters A, B, C, D, E, F, G and H contain solitary micrococcus strains mentioned in Table 8 (cf. p. 50), which could not be placed in any of the Groups I to XII.

The groups of »halophilic fermentative micrococci», »halotolerant fermentative micrococci» and »non-halotolerant fermentative micrococci» established through the Adansonian grouping of the investigated material could be further distinguished by the following properties:

»Halophilic fermentative micrococci» (Groups X; E and F):
— Pink pigmentation

»Halotolerant fermentative micrococci» (Groups I, II, III, IV, VIII, IX; C_1, C_2 and C_3):
— Optimum NaCl concentration for growth
— Acid production from arabinose
— Gelatine liquefaction
— Growth with urea as sole source of nitrogen

»Non-halotolerant fermentative micrococci» (Groups V, VI, VII, XI; D_1, D_2, D_3 and G):
— Pink pigmentation
— Nitrate reduction
— Gelatine liquefaction

VII. Fermentative micrococci; further subgrouping and description of the groups

As has been said before, the halotolerant and non-halotolerant groups of fermentative micrococci can be further subgrouped on the basis of certain characteristics. The following is a treatment of this further subgrouping, while at the same time the properties of all three groups shall be described. The subgroups that have been established will be denoted by the following symbols:

	Subgroups
Halophilic	
Halotolerant	
Non-halotolerant	

1. Halophilic fermentative micrococci

The group of »halophilic fermentative micrococci» comprises two subgroups. Subgroup ***a*** contains the pink pigment-forming strains No. 75 and 95x (= F, cf. Table 8, p. 50), while subgroup ***b*** consists of the strains forming no pink pigment, No. 244 and 245 (= Group X) and the strain No. 82 (= E) (cf. Fig. 6, p. 55 and Fig. 9, p. 70). As there were only three strains of the latter kind, further grouping was unnecessary.

The subgroups presented the following characteristcs (cf. Table 12, p. 66):

Sugbroup *a* (Strains No. 75 and 95x = F, cf. Table 8, p. 50)

Common characteristics: The bacteria reduced nitrate to nitrite, produced ammonia from peptone and grew with asparagin and urea as sole source of nitrogen; they were peroxidase-positive and oxidase-positive, produced acid from mannose, fructose, maltose, mannitol and sorbitol; they were facultatively anaerobic, formed pink pigment, grew in a medium containing 5, 10 and 15% NaCl and produced acid from glucose slightly under aerobic as well as

anaerobic conditions; their optimum pH range for growth was above pH 8.0, temperature about 30°C and concentration of NaCl about 5%.

Absent characteristics: The bacteria did not liquefy gelatine, nor did they hydrolyse casein; they were reductase-negative, lipolysis-negative and urease-negative; they did not produce acid from arabinose, xylose, galactose, saccharose, raffinose, dextrin, starch, glycogen, inulin and salicin; they caused no hydrolysis of starch and produced no acetoin; they did not grow without NaCl and caused no peptonisation in litmus milk.

As subgroup ***a*** only contained two strains, neither could be referred to as the most typical representative of this subgroup. The properties of these strains can be seen in Table 12, p. 66.

Subgroup *b* (Group X and strain No. 82 = E)

Common characteristics: The bacteria grew with asparagin and ammonium phosphate as sole source of nitrogen and they produced acid from mannose and maltose; they were facultatively anaerobic, caused hydrolysis of starch, grew in a medium containing 5, 10 and 15% NaCl and produced slightly acid from glucose under aerobic as well as anaerobic conditions; their optimum temperature range for growth was 20 to 30°C.

Other frequently present characteristics: Nitrate reduction; growth with urea as sole source of nitrogen; peroxidase and oxidase formation; growth on potato; optimum pH range for growth pH 7.0 to 8.0 and optimum concentration of NaCl 10%.

Absent characteristics: The bacteria did not liquefy gelatine, nor did they hydrolyse casein; they were lipolysis-negative and urease-negative; they produced no acid from arabinose, galactose, lactose, raffinose, dextrin, starch, glycogen, inulin, salicin and mannitol; they produced no acetoin and formed no pink pigment; they did not grow without NaCl and caused no changes in litmus milk.

The strain No. 245 could be considered the most typical representative of subgroup ***b*** (cf. Tables 12 and 13, pp. 66—67).

2. Halotolerant fermentative micrococci

The Adansonian Groups I, II, III, IV, VIII and IX could be mutually differentiated by their properties, shown in Table 10. The solitary strains, which have been denoted by C_1, C_2 and C_3 in Table

8 (p. 50), found their place among the above-mentioned groups in Table 10 on the basis of their properties (cf. Fig. 7).

Table 10. *Characteristics by which the group of halotolerant fermentative micrococci can be further subgrouped.*

	Number of strains displaying the characteristic								
Adansonian Group	I	II	III	IV	VIII	IX	C_1	C_2	C_3
Number of strains in the group	*28*	*80*	*11*	*3*	*4*	*2*	*6*	*3*	*3*
Characteristic:									
Optimum concentration of NaCl for growth, 5%	27	0	1	0	0	0	6	0	0
Acid from arabinose	26	78	1	0	0	2	3	3	0
Liquefaction of gelatine	12	44	11	0	4	0	4	2	0
Growth with urea as sole source of nitrogen	4	11	0	0	4	1	3	3	0

The differences between the groups revealed by Table 10 can be presented in the form of a diagram, and the following subgrouping system of the group »halotolerant fermentative micrococci» in Fig. 6 (p. 55) is then obtained (cf. Fig. 7).

The Adansonian Groups I and II differed mutually only in regard of their optimum concentration of NaCl for growth. The subgroups of the Groups I and II in Table 8 (p. 50) and the Groups II and IX could not be differentiated by any of the characteristics mentioned in the foregoing. The group of »halotolerant fermentative micrococci» would thus be divided into the subgroups $I + C_1$, $II + IX + C_2$, $IV + C_3$, VIII and III. These subgroups have been denoted with ***c***, ***d***, ***e***, ***f*** and ***g*** in Fig 7.

The subgroups shown in Fig. 7 presented the following characteristics (cf. Table 12, p. 66):

Subgroup *c* ($I + C_1$, cf. Table 8, p. 50)

Common characteristics: The bacteria produced acid from fructose and maltose, and grew in a medium containing 0, 5, 10 and 15% NaCl; they caused strong production of acid from glucose under aerobic conditions but mostly only slight acid production under anaerobic conditions; the optimum concentration of NaCl for growth was 5%.

Other frequently present characteristics: Nitrate reduction; production of ammonia from peptone; growth with ammonium tartrate and asparagin as sole source of nitrogen;

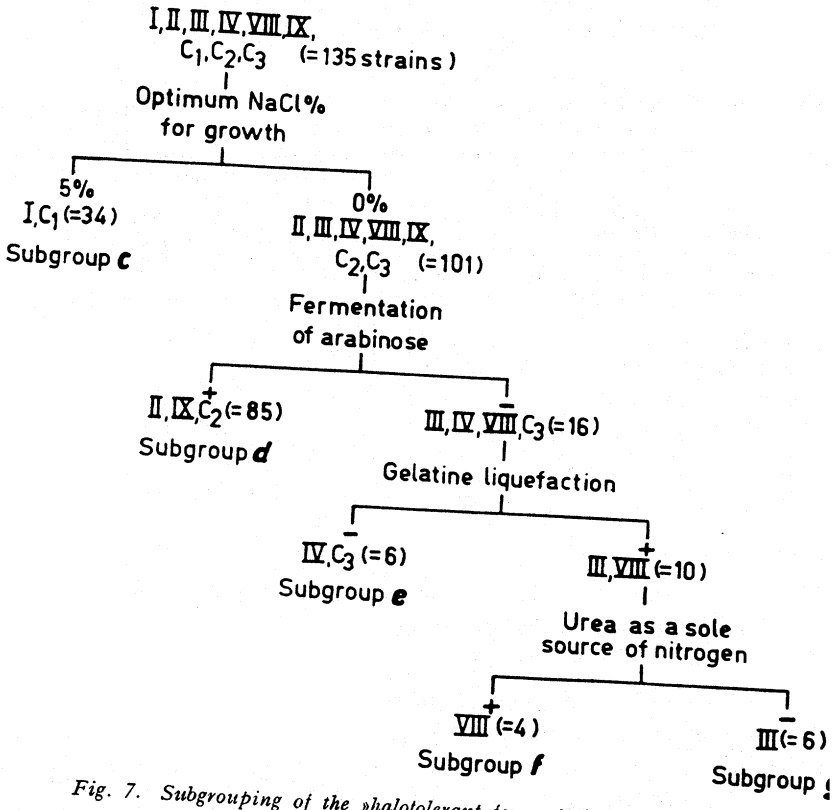

Fig. 7. Subgrouping of the »halotolerant fermentative micrococci.

acid production from arabinose, xylose, mannose, lactose, saccharose and dextrin; facultative anaerobes, less frequently aerobic growth; acid production in litmus milk; optimum pH range for growth pH 7.0 to 8.0 and optimum temperature range 20 to 30°C.

Absent characteristics: The bacteria did not produce acetoin, nor pink pigment, and did not cause separation of whey in litmus milk.

The strain No. 238 could be considered the most typical representative of subgroup **c** (cf. Tables 12 and 13, pp. 66—67).

Subgroup *d* (II + IX + C_2)

Common characteristics: The bacteria produced ammonia from peptone; they grew in a medium containing 0, 5, 10 and 15% NaCl and caused strong production of acid from glucose under aerobic conditions but mostly only slight production of acid under anaerobic conditions; they grew most strongly without NaCl.

Other frequently present characteristics: Nitrate reduction; growth with ammonium tartrate and asparagin as sole source of nitrogen; production of acid from arabinose, xylose, mannose, fructose, maltose, lactose, saccharose, dextrin and mannitol; facultative anaerobes, less frequently aerobic growth; acid production in litmus milk; optimum pH range for growth pH 7.0 to 8.0 and optimum temperature range mostly 20 to 30°C.

Absent characteristics: The bacteria did not produce acid from glycogen and caused no hydrolysis of starch; they did not produce acetoin, nor pink pigment, and did not cause peptonisation in litmus milk.

Strain No. 234 could be considered the most typical representative of subgroup ***d*** (cf. Tables 12 and 13, pp. 66—67).

Subgroup e (IV + C_3)

Common characteristics: The bacteria produced acid from mannose, fructose and maltose; they formed pigment and grew in a medium containing 0, 5, 10 and 15% NaCl; they caused strong production of acid from glucose under aerobic conditions but mostly only slight production of acid under anaerobic conditions; they grew most strongly without NaCl.

Other frequently present characteristics: Nitrate reduction; growth with ammonium tartrate, asparagin and ammonium phosphate as sole source of nitrogen; formation of reductase; acid production from xylose and dextrin; facultative anaerobes, less frequently aerobic growth; hydrolysis of starch; optimum pH range for growth pH 7.0 to 8.0 and optimum temperature range 20 to 30°C.

Absent characteristics: The bacteria did not grow with urea as sole source of nitrogen; they did not liquefy gelatine or cause hydrolysis of casein; they were peroxidase-negative and oxidase-negative; they produced no acid from arabinose, glycogen, inulin and salicin; they did not produce acetoin, nor pink pigment, and did not cause reduction, coagulation, peptonisation or separation of whey in litmus milk.

Strain No. 21 could be considered the most typical representative of subgroup ***e*** (cf. Tables 12 and 13, pp. 66—67).

Subgroup *f* (VIII)

Common characteristics: The bacteria reduced nitrate and produced ammonia from peptone; they grew with ammonium tartrate, asparagin, ammonium phosphate and urea as sole source of nitrogen; they liquefied gelatine and were peroxidase-positive and oxidase-positive; they grew on potato and produced acid from man-

nose, fructose and mannitol; they were facultatively anaerobic and grew in a medium containing 0, 5, 10 and 15% NaCl; they caused strong production of acid from glucose under aerobic conditions and only slight acid production under anaerobic conditions; they grew most strongly without NaCl.

Other frequently present characteristics: Formation of reductase; lipolysis; acid production from maltose, lactose, saccharose, salicin and glycerol; optimum pH range for growth pH 7.0 to 8.0 and above 8.0 and optimum temperature range 31 to 40°C.

Absent characteristics: The bacteria were urease-negative and produced no acid from arabinose, galactose, starch, glycogen or inulin; they caused no hydrolysis of starch, production of acetoin or formation of pink pigment; they caused no peptonisation in litmus milk.

Strain No. 192 could be considered the most typical representative of subgroup ***f*** (cf. Tables 12 and 13, pp. 66—67).

Subgroup *g* (III)

Common characteristics: The bacteria produced ammonia from peptone; they grew with ammonium tartrate as sole source of nitrogen and liquefied gelatine; they were reductase-positive; they grew on potato and produced acid from mannose, fructose, maltose, saccharose and dextrin; they were facultatively anaerobic and grew in a medium containing 0, 5, 10 and 15% NaCl; they produced acid in litmus milk and caused strong production of acid from glucose under aerobic conditions and mostly strong production of acid also under anaerobic conditions; their optimum pH range for growth was pH 7.0 to 8.0.

Other frequently present characteristics: Growth with asparagin as sole source of nitrogen; lipolysis; acid production from galactose and lactose; strongest growth without NaCl; optimum temperature range for growth 31 to 40°C.

Absent characteristics: The bacteria did not grow with urea as sole source of nitrogen; they were peroxidase-negative and oxidase-negative; they produced no acid from raffinose, glycogen or inulin; they caused no hydrolysis of starch, formed no pink pigment and caused no peptonisation in litmus milk.

Strain No. 104 could be considered the most typical representative of subgroup ***g*** (cf. Tables 12 and 13, pp. 66—67).

3. Non-halotolerant fermentative micrococci

The Adansonian groups V, VI, VII and XI can be mutually differentiated by the characteristics shown in Table 11. The strains forming pink pigment, all of which are so-called type strains obtained from the ATCC and NCTC collections, have not been taken into account in Table 11 and in Fig. 8. They have been referred here to subgroup ***h***. The solitary strains, denoted with D_1, D_2 and D_3 in Table 8 (p. 50), found their place among the above-mentioned groups on the basis of the characteristics stated in Table 11.

Table 11. *Characteristics by which the group of non-halotolerant fermentative micrococci can be further subgrouped.*

	Number of strains displaying the characteristic						
Adansonian group	V	VI	VII	XI	D_1	D_2	D_3
Number of strains in the group	*4*	*4*	*3*	*2*	*4*	*6*	*3*
Characteristic:							
Nitrate reduction	0	4	0	2	0	6	3
Gelatine liquefaction	4	4	3	0	4	0	3

The differences between the groups revealed by Table 11 could be presented in the form of a diagram, and the following subgrouping system of the group »non-halotolerant fermentative micrococci» was then obtained (Fig. 8; cf. also Fig. 9, p. 70).

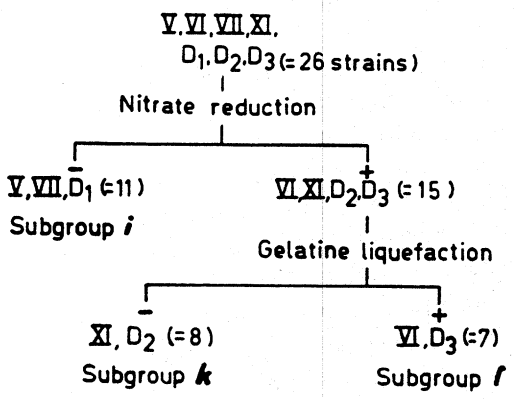

Fig. 8. Subgrouping of the »non-halotolerant fermentative micrococci».

The Adansonian groups V and VII could not be differentiated by any of the above-mentioned characteristics. The group of »non-halotolerant fermentative micrococci» forming no pink pigment could thus be subdivided into the subgroups V+VII + D_1, XI + D_2 and VI + D_3, which have been denoted with ***i***, ***k*** and ***l*** in Fig. 8.

The subgroups shown in Fig. 8 presented the following characteristics (cf. Tables 12, p. 66).

Subgroup *i* (V + VII + D_1, cf. Table 8, p. 50)

Common characteristics: The bacteria grew with ammonium tartrate and asparagin as sole source of nitrogen; they liquefied gelatine and grew in a medium containing 0 and 5% NaCl.

Other frequently present characterics: Ammonia production from peptone; growth with ammonium phosphate as sole source of nitrogen; acid production from mannose, fructose and maltose; facultative anaerobes; hydrolysis of starch; strong production of acid from glucose under aerobic conditions but only slight acid production under anaerobic conditions; growth in a medium containing 10% NaCl strongest growth without NaCl.

Absent characteristics: The bacteria did not reduce nitrate; they were peroxidase-negative and produced no acid from glycogen, salicin, mannitol, sorbitol or glycerol; they produced no acetoin and formed no pink pigment; they did not grow in a medium containing 15% NaCl and did not cause separation of whey in litmus milk.

Strain No. 24 could be considered the most typical representative of subgroup ***i*** (cf. Tables 12 and 13, pp. 66—67).

Subgroup *k* (XI + D_2)

Common characteristics: The bacteria reduced nitrate and produced acid from mannose and fructose; they were facultatively anaerobic and grew in a medium without NaCl.

Other frequently present characteristics: Growth with ammonium tartrate, asparagin and ammonium phosphate as sole source of nitrogen; formation of reductase; growth on potato; acid production from galactose and maltose; hydrolysis of starch; formation of pigment; strong production of acid from glucose under aerobic conditions but less strong acid production under anaerobic conditions; growth in a medium containing 5 and 10% NaCl and strongest growth without NaCl.

Absent characteristics: The bacteria did not grow with urea as sole source of nitrogen; they did not liquefy gelatine or cause hydrolysis of casein; they were peroxidase-negative and lipo-

lysis-negative; they produced no acetoin and no pink pigment: they did not grow in a medium containing 15 % NaCl and did not cause coagulation, peptonisation or separation of whey in litmus milk.

The strain No. 257 could be considered the most typical representative of subgroup ***k*** (cf. Tables 12 and 13, pp. 66—67).

Subgroup *l* (VI + D_3)

Common characteristics: The bacteria reduced nitrate and grew with ammonium tartrate and asparagin as sole source of nitrogen; they liquefied gelatine and grew in a medium containing 0 and 5 % NaCl.

Other frequently present characteristics: Ammonia production from peptone; growth with ammonium phosphate as sole source of nitrogen; hydrolysis of casein; formation of reductase; formation of oxidase; growth on potato; acid production from mannose and fructose and hydrolysis of starch; facultative anaerobes; growth in a medium containing 10 % NaCl; acid production from glucose in approximately equal intensity under aerobic and anaerobic conditions; optimum pH range for growth pH 6.0 to 6.9 and strongest growth without NaCl.

Absent characteristics: The bacteria were lipolysis-negative and urease-negative; they produced no acid from raffinose, starch, inulin, salicin, sorbitol or glycerol; they produced no acetoin and no pink pigment, and they did not grow in a medium containing 15 % NaCl.

Strain No. 185 could be considered the most typical representative of subgroup ***l*** (cf. Tables 12 and 13, pp. 66—67).

Table 12. *Characteristics of the different subgroups of the groups of halophilic, halotolerant and non-halotolerant fermentative micrococci.*

Characteristic \ Subgroup	Number of strains displaying the characteristic									
	Halophilic group		Halotolerant group					Non-halotolerant group		
	a	**b**	**c**	**d**	**e**	**f**	**g**	**i**	**k**	**l**
Number of strains in the subgroup	2	3	34	85	6	4	11	11	8	7
Nitrate reduction	**2**	2	33	83	5	**4**	4	0	**8**	**7**
Ammonia production from peptone	**2**	1	31	**85**	1	**4**	**11**	10	4	5
Growth with ammonium tartrate as sole source of N	1	1	30	81	5	**4**	**11**	**11**	7	**7**
» » asparagin » » » » »	**2**	**3**	31	80	5	**4**	10	**11**	6	**7**
» » ammonium phosphate » » » » »	1	**3**	5	21	4	**4**	5	9	6	6
» » urea » » » » »	**2**	2	7	15	0	**4**	0	3	0	1
Gelatine liquefaction	0	0	16	46	0	**4**	**11**	**11**	0	**7**
Casein hydrolysis	0	0	2	4	0	2	3	7	0	6
Reductase activity	0	1	17	42	4	3	**11**	6	6	6
Peroxidase »	**2**	2	4	3	0	**4**	0	0	0	3
Oxidase »	**2**	2	3	3	0	**4**	0	4	1	5
Lipolytic »	0	0	7	18	1	3	9	1	0	0
Urease »	0	0	21	40	3	0	7	1	1	0
Growth on potato	1	2	8	45	3	**4**	**11**	10	7	5
Acid from arabinose	0	0	29	83	0	0	1	1	4	1
» » xylose	0	1	33	83	5	2	2	4	4	2
» » mannose	**2**	**3**	33	83	**6**	**4**	**11**	10	**8**	6
» » galactose	0	0	19	57	3	0	10	2	7	4
» » fructose	**2**	1	**34**	84	**6**	**4**	**11**	10	**8**	6
» » maltose	**2**	**3**	**34**	83	**6**	3	**11**	9	6	3
» » lactose	1	0	31	83	2	3	10	3	4	1
» » saccharose	0	1	32	81	3	3	**11**	8	5	3
» » raffinose	0	0	14	32	1	2	0	2	4	0
» » dextrin	0	0	29	73	5	2	**11**	6	4	4
» » starch	0	0	2	3	3	0	2	1	3	0
» » glycogen	0	0	1	0	0	0	0	0	3	1
» » inulin	0	0	4	10	0	0	0	1	4	
» » salicin	0	0	14	34	0	3	1	0	4	
» » mannitol	**2**	0	25	72	2	**4**	6	0	3	
» » sorbitol	**2**	1	21	51	2	2	1	0	2	0
» » glycerol	1	1	16	50	1	3	6	0	5	0
Aerobic	0	0	1	15	1	0	0	2	0	1
Facultatively anaerobic	**2**	**3**	33	70	5	**4**	**11**	9	**8**	6
Starch hydrolysis	0	**3**	1	0	4	0	0	9	6	5
Acetoin production	0	0	0	0	0	0	5	0	0	0
Pigmentation	0	1	15	22	**6**	2	2	9	6	4
Pink pigmentation	**2**	0	0	0	0	0	0	0	0	0
Growth at NaCl concentration 0 %	0	0	**34**	**85**	**6**	**4**	**11**	**11**	**8**	**7**
» » » » 5 %	**2**	**3**	**34**	**85**	**6**	**4**	**11**	**11**	7	**7**
» » » » 10 %	**2**	**3**	**34**	**85**	**6**	**4**	**11**	7	5	6
» » » » 15 %	**2**	**3**	**34**	**85**	**6**	**4**	**11**	0	0	0
Litmus milk test, acid production	1	0	28	75	1	2	**11**	1	2	2
» » » reduction	1	0	6	17	0	1	2	4	2	1
» » » coagulation	1	0	4	4	0	1	2	5	0	1
» » » peptonisation	0	0	1	0	0	0	0	4	0	1
» » » separation of whey	1	0	0	1	0	1	1	0	0	1
Acid production from glucose, aerobic pH 7.3—6.3	**2**	**3**	0	0	0	0	0	1	1	3
» » » » » pH<6.3	0	0	**34**	**85**	**6**	**4**	**11**	10	7	4
» » » » anaerobic pH 7.3—6.3	**2**	**3**	33	81	5	**4**	3	10	5	5
» » » » » pH<6.3	0	0	1	4	1	0	8	1	3	2
Optimum pH range for growth 6.0—6.9	0	0	2	3	1	0	0	5	3	5
» » » » » 7.0—8.0	0	2	30	72	5	2	**11**	6	4	2
» » » » » >8.0	**2**	1	2	10	0	2	0	0	1	0
Optimum temperature for growth 20—30°C	**2**	**3**	29	64	4	1	1	5	5	3
» » » » 31—40°C	0	0	5	21	2	3	10	6	3	3
» » » » >40°C	0	0	0	0	0	0	0	0	0	1
Optimum NaCl concentration for growth 0 %	0	0	0	**85**	**6**	**4**	**11**	10	7	6
» » » » » 5 %	**2**	1	**34**	0	0	0	1	1	1	1
» » » » » 10 %	0	2	0	0	0	0	0	0	0	0
» » » » » 15 %	0	0	0	0	0	0	0	0	0	

ble 13. *Characteristics of the most typical strains representing each subgroup of the groups of halophilic, halotolerant and non-halotolerant fermentative micrococci.*

Characteristic / Subgroup	Halophilic group	Halotolerant group					Non-halotolerant group		
	b	*c*	*d*	*e*	*f*	*g*	*i*	*k*	*l*
Representative strain No.	245	238	234	21	192	104	24	257	185
Nitrate reduction	+	+	+	+	+	−	−	+	+
Ammonia production from peptone	−	+	+	−	+	+	+	−	+
Growth with ammonium tartrate as sole source of N	−	+	+	+	+	+	+	+	+
» » asparagin » » » » »	+	+	+	+	+	+	+	+	+
» » ammonium phosphate » » » » »	+	+	+	+	+	+	+	+	+
» » urea » » » » »	+	−	−	−	+	−	−	−	−
Gelatine liquefaction	−	−	−	−	+	+	+	−	+
Casein hydrolysis	−	−	−	−	+	−	+	−	+
Reductase activity	−	−	+	+	+	+	+	−	+
Peroxidase »	+	−	−	−	+	−	−	−	−
Oxidase »	+	−	−	−	+	−	−	−	−
Lipolytic »	−	−	−	−	+	+	+	−	−
Urease »	−	+	+	−	−	+	+	−	−
Growth on potato	−	−	+	−	+	+	+	+	+
Acid from arabinose	−	+	+	−	−	−	−	+	−
» » xylose	−	+	+	+	−	−	+	+	−
» » mannose	+	+	+	+	+	+	+	+	−
» » galactose	−	−	+	+	−	+	−	+	+
» » fructose	−	+	+	+	+	+	+	+	+
» » maltose	+	+	+	+	+	+	+	+	+
» » lactose	−	+	+	+	+	+	−	+	+
» » saccharose	−	+	+	+	+	+	+	+	+
» » raffinose	−	−	+	−	+	−	−	+	−
» » dextrin	−	+	+	+	+	+	+	+	+
» » starch	−	−	−	+	−	−	−	+	−
» » glycogen	−	−	−	−	−	−	−	+	−
» » inulin	−	−	−	−	−	−	−	+	−
» » salicin	−	−	−	−	+	−	−	+	−
» » mannitol	−	+	+	−	+	+	−	+	−
» » sorbitol	−	+	+	−	+	−	−	+	−
» » glycerol	−	+	+	−	+	+	−	+	−
Aerobic	−	−	−	−	−	−	−	−	−
Facultatively anaerobic	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	−	−	+	−	−	+	+	+
Acetoin production	−	−	−	−	−	+	−	−	−
Pigmentation	+	+	−	+	+	−	+	+	+
Pink pigmentation	−	−	−	−	−	−	−	−	−
Growth at NaCl concentration 0 %	−	+	+	+	+	+	+	+	+
» » » » 5 %	+	+	+	+	+	+	+	+	+
» » » » 10 %	+	+	+	+	+	+	−	+	+
» » » » 15 %	+	+	+	+	+	+	−	−	−
Litmus milk test, acid production	−	+	−	+	+	+	−	−	+
» » » reduction	−	−	−	−	+	+	+	+	−
» » » coagulation	−	−	−	−	+	+	+	−	−
» » » peptonisation	−	−	−	−	−	−	+	−	−
» » » separation of whey	−	−	−	−	+	+	−	−	−
Acid production from glucose, aerobic pH 7.3—6.3	+	−	−	−	−	−	−	−	−
» » » » » pH<6.3	−	+	+	+	+	+	+	+	+
» » » » anaerobic pH 7.3—6.3	+	+	+	+	+	−	+	+	+
» » » » » pH<6.3	−	−	−	−	−	+	−	−	−
Optimum pH range for growth 6.0—6.9	−	−	−	−	−	−	+	−	+
» » » » » 7.0—8.0	+	+	+	+	+	+	+	+	−
» » » » » >8.0									
Optimum temperature for growth 20—30°C	+	+	+	+	−	−	+	−	−
» » » » 31—40°C	−	−	−	−	+	+	−	+	+
» » » » >40°C									
Optimum NaCl concentration for growth 0 %	−	−	+	+	+	+	+	+	+
» » » » » 5 %	−	+	−	−	−	−	−	−	−
» » » » » 10 %	+	−	−	−	−	−	−	−	−
» » » » » 15 %	−	−	−	−	−	−	−	−	−

VIII. Discussion

The experimental material concerned in the present investigation comprised 171 strains of micrococci isolated from dry sausages and from brines used in the manufacturing of dry sausages and hams, and 20 type strains obtained from the American Type Culture Collection, the National Collection of Type Cultures (London) and the Laboratorium voor Microbiologie, Technische Hogeschool (Delft, Holland). An attempt was made to group and to identify these micrococci by the grouping systems given in Bergey's Manuals (1948, 1957) and by those presented by SHAW et al. (1951) and by HILL (1959).

It was found that identification of meat micrococci in accordance with the systems in Bergey's Manuals rather fails its purpose, whereas the grouping system presented by SHAW et al. provides a more clear-cut basis for the grouping and identification of these micrococci. According to investigations carried out by GREGORY and MABBIT (1957 b), the *Staphylococcus lactis* group of SHAW et al. comprises various kinds of organisms, and it should be possible to subdivide this group further. Likewise, HILL (1959) has noted in his work of grouping the micrococci by the so-called Adansonian method of classification, developed by SNEATH (1957 b), that the *S. lactis* group of SHAW et al. is heterogeneous.

In the author's investigations the micrococci have been grouped also in accordance with the Adansonian classification procedure developed by SNEATH, with the result that nearly all meat micrococci were observed to find their place in the said *S. lactis* group. It seems rather obvious, therefore, that the treatment of all such bacteria as belonging to one single species is unsatisfactory from the viewpoint of meat technology, seeing that a great number of distinctly different micrococci would fall under this species appellation. In particular, it would include a considerable variety of different micrococci typical of meat and meat products.

SHAW et al. (1951) refer all aerobic, Gram-positive, catalase-positive cocci to genus *Staphylococcus*, which comprises five species, viz., *S.*

aureus, *S. saprophyticus*, *S. lactis*, *S. roseus* and *S. afermentans*. According to HILL (1959), three of the five species (*S. aureus*, *S. saprophyticus* and *S. roseus*) are natural groups, but in his opinion this is not true of *S. lactis* and *S. afermentans*. HILL suggests that *S. roseus* and *S. afermentans* should be referred to genus *Micrococcus*. He calls the groups *S. lactis* and *S. afermentans* of SHAW et al. »fermentative miscellaneous staphylococci» and »non-fermentative miscellaneous micrococci», respectively. However, the group of »fermentative miscellaneous micrococci» in the system presented by HILL does not include the strains forming pink pigment; *M. roseus*, though fermentative, would thus be excluded from this group. In the present work also strains forming pink pigment have been considered as belonging to the group of fermentative micrococci.

It is a much-debated question whether the genus of the bacteria under consideration should be called *Micrococcus* or *Staphylococcus*. The most appropriate solution seems to be, in the author's opinion, to retain both genera, drawing the line between them as follows: *S. aureus* and *S. saprophyticus* of SHAW et al. would be referred to genus *Staphylococcus*, and all other to genus *Micrococcus*. The latter are further subgrouped into fermentative and non-fermentative micrococci. The differences between the grouping systems suggested by SHAW et al., by HILL and by the present author are illustrated by the following tabulation:

SHAW et al. (1951)	HILL (1959)	Present Author (1960)
Staphylococcus:	*Staphylococcus:*	*Staphylococcus:*
S. aureus	*S. aureus*	*S. aureus*
S. saprophyticus	*S. saprophyticus*	*S. saprophyticus*
		Micrococcus:
S. lactis	Fermentative (miscellaneous staphylococci)	Fermentative: 1. Halophilic; subgroups **a**, **b** 2. Halotolerant; subgroups **c**, **d**, **e**, **f**, **g** 3. Non-halotolerant; subgroup **h** = *M. roseus* subgroups **i**, **k**, **l**
	Micrococcus:	
S. roseus	*M. roseus*	
S. afermentans	Non-fermentative (miscellaneous micrococci)	Non-fermentative

In the author's investigations the meat micrococci could be divided into Adansonian groups by means of SNEATH's (1957 b) method. The

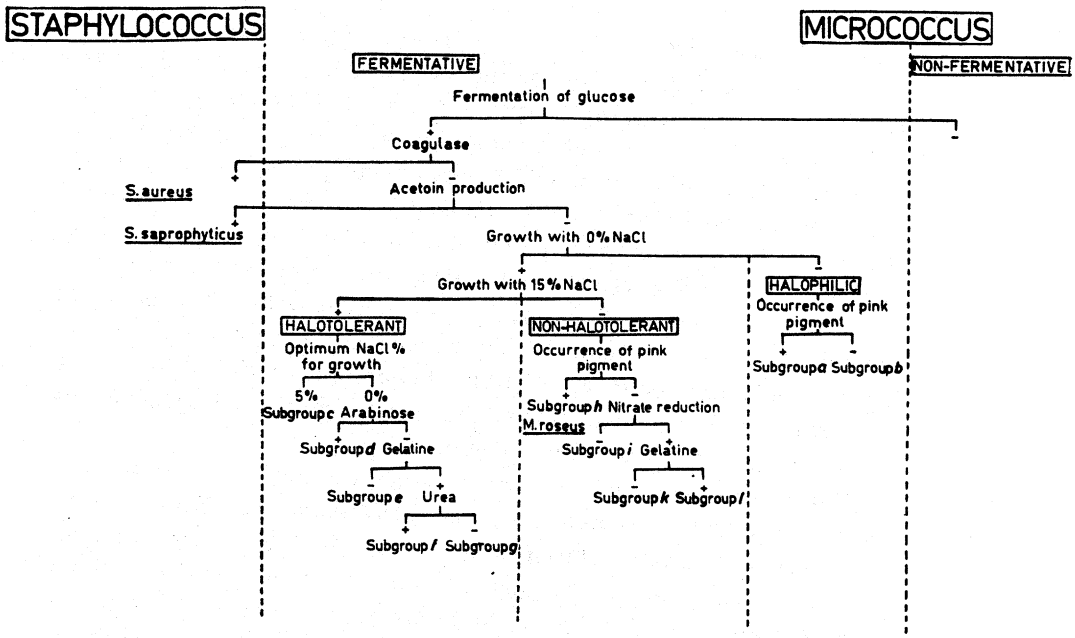

Fig. 9. Grouping system of the micrococci.

fermentative micrococci (i.e., the *S. lactis* group of SHAW et al., or HILL's »fermentative miscellaneous staphylococci») could then be subgrouped into the groups of halophilic, halotolerant and non-halotolerant micrococci. As can be seen from Fig. 9, these groups could further be separated into eleven subgroups (***a***, ***b***, ***c***, ***d***, ***e***, ***f***, ***g***, ***h***, ***i***, ***k*** and ***l***).

It was seen that the meat micrococci mainly belonged to the groups of halotolerant and non-halotolerant fermentative micrococci. The selection procedure for micrococci, developed by the author in his previous investigations (POHJA 1960, cf. also pp. 18—20), has been used successfully in application to a series of 702 strains of micrococci, among which three strains could be singled out by the procedure as being suitable for use in the manufacturing of dry sausages. In the classification system shown in Fig. 9, these three strains find their places in subgroups ***c*** and ***d*** of the group of »halotolerant fermentative micrococci». It can be stated as an inference from this observation that the grouping presented in Fig. 9 and the selection procedure outlined on p. 18 can be employed in finding suitable strains of micrococci for technological use in the manufacturing of dry sausages. If the material in hand consists of micrococci already grouped in

accordance with Fig. 9, only such strains belonging to the group of »halotolerant fermentative micrococci» need be subjected to examination by the selection procedure which grow at 20°C in a medium containing 5% NaCl and having pH about 6.0. If, on the other hand, an unknown, not previously grouped set of strains has to be examined, the grouping in accordance with Fig. 9 is unnecessary, as the selection procedure can be applied immediately to the original material. In this case, too, the first step is to eliminate the strains which do not grow at 20°C on a substrate with 5% NaCl content and pH about 6.0.

The micrococci investigated by SHAW et al. and by HILL seem to differ rather greatly from the micrococci occurring in meat products and in brines in respect of their properties. However, this constitutes no reason to establish a particular grouping and identification system for meat micrococci. As SHAW et al. have pointed out, every type of bacteriologist should not be allowed to develop his own classification for cocci isolated from sources of a given kind; rather should an attempt be made to classify within one system the group as a whole. The Adansonian method of classification presented by SNEATH should therefore be applied to the investigation of cocci isolated from the greatest possible variety of sources and an investigation should be made to find out how the groups obtained in this manner will be placed in the systems of SHAW et al. and of HILL, and in the system that has been supplemented as shown in this work. The result would evidently be a grouping of the entire group of micrococci surpassing all those suggested heretofore in appropriateness and exactitude.

The value of the present results from the viewpoint of applications in the field of meat technology will obviously depend on the fact fhat the investigated micrococci represent as great a variety of micrococci typical of meat products as can reasonably be desired. The possibility should also be kept in mind that the properties tested in this work fail to include some characteristics which might have brought the groups and their mutual differences into better relief. On the other hand, it is thought to be very difficult in bacteriological investigations of this kind to obtain an experimental material of greatest conceivable variety. However, in view of the fact that the micrococci selected to constitute the objects of the present investigation had been isolated from several kinds of dry sausages and brines originating in several factories, it seems reasonable to assume that they represent a good cross section of the typical micrococci occurring in meat products and in curing brines.

Summary

1. The grouping of 171 strains of micrococci derived from meat products and curing brines and of 20 strains obtained from the American Type Culture Collection, the National Collection of Type Cultures (London) and the Laboratorium voor Microbiologie, Technische Hogeschool (Delft, Holland) has been investigated.

2. It was observed that the majority of the meat micrococci found their place in the *Staphylococcus lactis* group of SHAW et al. (1951). Application of SNEATH's (1957a, b) Adansonian classification system revealed that 136 of the 171 strains of meat micrococci could be placed in 10 groups, all representing the said *S. lactis* group; the exception was a group comprising 11 strains, of which five represented the *S. saprophyticus* group of SHAW et al. Thirty strains of meat micrococci belonged to no Adansonian group, but 23 of them could best be identified as *S. lactis* according to SHAW et al.

3. The ten Adansonian groups could be differentiated by the ability of the bacteria to grow in media with 0 and 15% NaCl, respectively. On the basis of this, they have been considered to constitute three distinct groups, which have been referred to as »halophilic fermentative micrococci», »halotolerant fermentative micrococci» and »non-halotolerant fermentative micrococci».

4. Of the species distinguished by SHAW et al., only *S. aureus* and *S. saprophyticus* have been referred to genus *Staphylococcus* in this work, and all strains of other types have been considered as belonging to genus *Micrococcus*. The grouping system suitable for identification of the latter kind of bacteria could therefore include the species *S. roseus* of SHAW et al., HILL's (1959) group of »non-fermentative miscellaneous micrococci» and the three new groups described in this work.

5. According to the results of the present work, the micrococci could be grouped into fermentative and non-fermentative micrococci. The fermentative micrococci were further subdivided into the

halophilic, halotolerant and non-halotolerant groups. The group of »halophilic fermentative micrococci» was divided into two subgroups (***a*** and ***b***) on the basis of the formation of pink pigment. The group of »halotolerant fermentative micrococci» could be divided into five subgroups (***c***, ***d***, ***e***, ***f*** and ***g***) by the following characteristics: optimum NaCl concentration for growth, acid production from arabinose, liquefaction of gelatine, and growth with urea as sole source of nitrogen. The group of »non-halotolerant fermentative micrococci» could be divided into four subgroups (***h***, ***i***, ***k*** and ***l***) by the formation of pink pigment, reduction of nitrate and liquefaction of gelatine. Subgroup ***h*** contains *M. roseus* (*S. roseus* of SHAW et al.) because this species has to be counted among the fermentative bacteria.

6. No attempt at naming any of the above-mentioned subgroups has been made in this work.

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SELOSTUS:

TUTKIMUKSIA KESTOLIHATUOTTEIDEN MIKROKOKKIEN LUOKITTELUSTA JA KUVAILUSTA

Selostettava tutkimus on suoritettu Osuusteurastamojen Tutkimuslaitoksessa Hämeenlinnassa vv. 1958—1960. Tämän tutkimuksen tarkoituksena on ollut luoda perustaa kestolihatuotteiden mikrokokkien ryhmittelylle ja valaista mm. seuraavia kysymyksiä:

(a) Onko lihajalosteista peräisin olevissa mikrokokeissa sellaisia tyyppejä, joita ei aikaisemmin ole kuvailtu?

(b) Jos lihamikrokokit voidaan ryhmitellä tarkoituksenmukaisella tavalla, niin miten tämä ryhmittely soveltuu mikrokokkien ryhmittelyperusteista aikaisemmin esitettyihin käsityksiin?

Koeaineistoksi valittiin tekijän aikaisempien tutkimusten yhteydessä eristettyjen 702:n mikrokokkikannan joukosta 171 kantaa, jotka kaikki poikkesivat toisistaan jonkin ominaisuuden perusteella. Lisäksi koeaineisto käsitti 20 kansainvälisistä kokoelmista saatua kantaa (vrt. sivu 22). Mikrokokkikannat yritettiin ensin ryhmitellä Bergeyn käsikirjoissa (1948, 1957) esitettyjen, sekä SHAW, STITT ja COWANin (1951) ja HILLin (1959) systeemien mukaan ja lopulta koeaineistoon sovellettiin SNEATHin (1957a, b) kehittämää ns. adansonilaista luokittelutapaa. Tämän antamien tulosten perusteella yritettiin täydentää SHAW, STITT ja COWANin ja HILLin esittämiä mikrokokkien ryhmittelysysteemejä siten, että ne sulkisivat sisäänsä myös »lihamikrokokit».

Näin laadittuun ryhmittelysysteemiin on lopuksi sijoitettu tekijän aikaisempien tutkimusten yhteydessä 702:n mikrokokkikannan joukosta valitut kolme kantaa, joiden voitiin katsoa sopivan kestomakkaran valmistuksessa käytettäviksi. Tarkoituksena on ollut osoittaa, muodostavatko nämä hyödylliset mikrokokit oman alaryhmänsä laaditussa ryhmittelysysteemissä. Tärkeimmät tutkimustulokset olivat seuraavat:

1. Lihamikrokokkien identifiointi Bergeyn käsikirjojen mukaan näyttää olevan varsin epätarkoituksenmukaista. SHAW, STITT ja COWANin (1951) esittämä ryhmittelykaavio sitä vastoin antanee selväpiirteisemmän perustan kokkien ryhmittelemiseksi ja identifioimiseksi kuin Bergeyn käsikirjojen mukaiset ryhmittelytavat. HILL on todennut ryhmitellessään mikrokokkeja SNEATHin kehittämän ns. adansonilaisen luokittelutavan mukaan, että SHAW, STITT ja COWANin *Staphylococcus lactis*-ryhmä on epäyhtenäinen. Tekijän suorittamissa tutkimuksissa mikrokokit ryhmiteltiin myös adansonilaisen luokittelutavan mukaan.

Sen antamien tulosten perusteella todettiin, että jokseenkin kaikki lihamikrokokit sijoittuivat *S. lactis*-ryhmään. Tekijän mielestä niiden käsittely yhtenä ainoana lajina lihateknologian kannalta ei ole tarkoituksenmukaista, koska ko. lajin nimen piiriin tässä esitettyjen tutkimusten mukaan sijoittuu suuri määrä toisistaan selvästi poikkeavia mikrokokkeja ja nimenomaan sellaisia, jotka ovat lihalle ja lihajalosteille tyypillisiä.

2. SHAW, STITT ja COWAN (1951) lukevat kaikki aerobiset grampositiiviset kokit *Staphylococcus*-sukuun kuuluviksi, joka käsittää viisi lajia, *S. aureus, S. saprophyticus, S. lactis, S. roseus* ja *S. afermentans*. HILLin (1959) mukaan em. viidestä lajista *S. aureus, S. saprophyticus* ja *S. roseus* ovat luonnollisia ryhmiä, mutta lajit *S. lactis* ja *S. afermentans* eivät niitä olisi. HILL ehdottaa, että lajit *S. roseus* ja *S. afermentans* olisi käsitettävä sukuun *Micrococcus* kuuluviksi. SHAW, STITT ja COWANin ryhmästä *S. lactis* HILL käyttää termiä »fermentoivat sekalaiset stafylokokit» (»fermentative miscellaneous staphylococci») ja *S. afermentans*-ryhmästä termiä »ei fermentoivat sekalaiset mikrokokit» (»non-fermentative miscellaneous micrococci»). HILLin esittämässä kaaviossa ryhmään »fermentoivat sekalaiset mikrokokit» eivät kuitenkaan kuulu punaista pigmenttiä muodostavat kannat, joten *M. roseus*, vaikka se on fermentoiva, ei kuuluisi tähän ryhmään. Tästä huolimatta tässä esitettävässä tutkimuksessa on katsottu fermentoivien ryhmään kuuluviksi myös punaista pigmenttiä muodostavat kannat.

3. Vaikka on kiistanalaista, onko suvun nimenä käytettävä nimeä *Micrococcus* vai *Staphylococcus*, tekijä on pitänyt parhaiten oikeaan osuvana seuraavaa rajanvetoa: SHAW, STITT ja COWANin *S. aureus* ja *S. saprophyticus* luetaan kuuluviksi *Staphylococcus*- ja kaikki muut *Micrococcus* sukuun.

4. Suoritetussa tutkimuksessa lihamikrokokit voitiin jakaa adansonilaisiin ryhmiin SNEATHin (1957b) menetelmän mukaan. Tällöin havaittiin 171:stä lihamikrokokkikannasta 136 kannan muodostavan 10 ryhmää. Kaikki ryhmät olivat sijoitettavissa em. *S. lactis*-ryhmään, lukuunottamatta 11 kannan muodostamaa ryhmää, mistä 5 kantaa oli sijoitettavissa SHAW, STITT ja COWANin *S. saprophyticus*-ryhmään. 30 lihamikrokokkikantaa ei kuulunut mihinkään adansonilaiseen ryhmään, mutta näistä 23 kantaa oli SHAW, STITT ja COWANin mukaan identifioitavissa lajiksi *S. lactis* (vrt. taulukko 8, sivu 50).

5. Kymmenen adansonilaista ryhmää voitiin erottaa toisistaan sen perusteella, kasvoivatko ko. bakteerit 0 % ja/tai 15 % NaCl:a sisältävillä ravintoalustoilla. Tämän perusteella katsottiin näiden muodostavan kolme ryhmää, nimittäin »suolaa vaativat fermentoivat mikrokokit» (»halophilic fermentative micrococci), »suolaa sietävät fermentoivat mikrokokit» (»halotolerant fermentative micrococci») ja »suolaa karttavat fermentoivat mikrokokit» (»non-halotolerant fermentative micrococci»).

6. Esitetyssä tutkimuksessa on sukuun *Staphylococcus* sisällytetty SHAW, STITT ja COWANin lajeista vain *S. aureus* ja *S. saprophyticus* ja muuntyyppiset mikrokokkikannat on käsitetty sukuun *Micrococcus* kuuluviksi. Viimeksi mainittujen mikrokokkien ryhmittelyyn ja identifiointiin soveltuva luokittelusysteemi saattaisi siis käsittää SHAW, STITT ja

COWANin lajin *S. roseus*, HILLin ryhmän »ei fermentoivat sekalaiset mikrokokit» sekä edellämainitut kolme uutta ryhmää.

7. Ryhmä »suolaa vaativat fermentoivat mikrokokit» jaettiin punaisen pigmentin muodostuksen perusteella kahdeksi alaryhmäksi (***a*** ja ***b***). Ryhmä »suolaa sietävät fermentoivat mikrokokit» voitiin jakaa viideksi alaryhmäksi (***c***, ***d***, ***e***, ***f*** ja ***g***) seuraavien ominaisuuksien perusteella: kasvulle optimaalinen NaCl-konsentraatio, haponmuodostus arabinoosista, gelatiinin liuotus ja kasvu virtsa-aineen ollessa ainoana N-lähteenä. Ryhmä »suolaa karttavat fermentoivat mikrokokit» voitiin punaisen pigmentin muodostuksen, nitraatin pelkistyksen ja gelatiinin liuotuksen perusteella jakaa neljäksi alaryhmäksi (***h***, ***i***, ***k*** ja ***l***.).

8. Tekijän suorittamissa aikaisemmissa tutkimuksissa (POHJA 1960) kehitetyn mikrokokkien valitsemissysteemin avulla löydetyt kolme kestomakkaran valmistukseen sopivaa kantaa voitiin sijoittaa ryhmän »suolaa sietävät fermentoivat mikrokokit» alaryhmiin ***c*** ja ***d***.